Seasonal variation in bee venom extraction and its effect on *Apis mellifera* L. colony performance

BY

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CERTIFICATE-I

This is to certify that this thesis entitled, **"Seasonal variation in bee venom extraction and its effect on *Apis mellifera* L. colony performance**", submitted for the degree of **Master of Science** in the subject of **Agricultural Entomology** to the **Chaudhary Charan Singh Haryana agricultural University, Hisar**, is a bonafide research work carried out by **Ms. N. Aparna (2019A48M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE-II

This is to certify that this thesis entitled, **"Seasonal variation in bee venom extraction and its effect on *Apis mellifera* L. colony performance**", submitted by **Ms. N. Aparna (2019A48M)**, to the **Chaudhary Charan Singh Haryana Agricultural University, Hisar** , in partial fulfillment of the requirements for the degree of "**Master of Science**" in the subject of **Entomology** has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

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Needless to say, errors and omissions, if any, are all mine.

Hisar
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LIST OF ABBREVIATIONS

mg	Milligram
kg	Kilogram
V	Volt
AC	Alternative current
g	Gram
%	Percent
DC	Direct current
S	Second
Sq.	Square
cm	Centimeter
Min.	Minutes
µg	Microgram
ml	Millilitre
LD_{50}	Lethal dose for 50% mortality
A	Ampere
^0C	Degree celsius
mm	Millimeter
ANOVA	Analysis of variance
RBD	Randomised block design
SMW	Standard meteorological weeks
CD	Critical difference
NS	Non significant
SE (m)	Standard error (mean)
DAE	Days after extraction
Viz.	Videlicet (namely)

CHAPTER- I

INTRODUCTION

Among the Hymenopterans, honey bees, belonging to family Apidae are one of the world's most beneficial insect playing a critical role in many terrestrial ecosystems. Honeybees are special friend to mankind because of its participation in pollination services as well as production of many cherishable bee-products such as honey, royal jelly, bee pollen, bees wax, propolis, bee venom, etc. Among different honeybee species found in India, the European honeybee, *Apis mellifera* is the most common domesticated species because of its docile nature. *A. mellifera* is not aggressive to human beings but sometimes its envenoming nature cause trouble to beekeepers when they feel threatened. This bee venom, produced by bees as a defensing mechanism, is one such bee-product that can boost economy of beekeeper in both dearth and honey flow period.

Bee venom can be described as an odourless, colourless, clear watery liquid with pungent smell, bitter taste and slightly acidic pH (4.5 to 5.5) (Devi *et al*., 2016; El-saeady *et al*., 2016; Ali, 2012). It appears as yellow powder in dried form and turns brown in presence of oxygen. It consists of some volatile compounds and is soluble in water but insoluble in alcohol. It becomes prone to destruction if exposed to sun light, high temperatures (Bogdanov, 2016). It is a complex mixture of peptidyl toxins and enzymes. Melittin and apamin are two important biologically active peptides responsible for toxicity of bee venom as they mainly act on phospholipid bilayer membrane. Hyaluronidase and phopholipase A2 are the enzymes responsible for strong immune response created by enemy's body (Onari *et al*., 2016).

Bee venom is the release of an exocrine gland i.e. the venom gland or acid gland as a defensive response.. Poison is secreted from branched secretory cells of venom gland and stored in poison sac. When honey bee experiences any external danger stimuli, venom release occurs through the stinging apparatus. This sting apparatus consists of a stylus, two barbed hollow lancets on both side of stylus and a basal bulb. The honey bee doesn't push the sting into enemy's body, it goes deeper with the movement of barbed structures which penetrates the sting deeper and prevents retraction (Bogdanov, 2016). This sting shaft is 2·0 mm long, 1.0 mm dia. with the barbs each 0.03 mm long (Benton *et al*., 1963). Along with the produce of venom gland, bee venom also has the produce of other exocrine glands, such as alarm pheromones isopentyl acetate (IPA) from koschevnikov gland and 2-heptanone (2HPT) from mandibular gland which is associated with the sting apparatus (Collins *et al.,* 1989; Onari *et al*., 2016; Shearer and Boch, 1965; Free *et al*., 1983).

Only queen and worker castes can produce bee venom as the venom gland or acid gland, associated with bee venom production, is a modified ovipositor. Young bees do not produce bee venom. Production of bee venom starts rising when bee is of 2-3 days old after emergence and attains its peak when the worker bee engages itself as guard bee and forager bee (Roat *et al.,* 2006) It decreases as the bee gets older. The queen bee's production of venom is highest on emergence to compete with other queens.

So far, various methods of bee venom extraction have been performed in abroad and also in India. The most primitive method of extraction is the manual extraction of bee venom by cutting the venom sac. This method was not quite accepted because of the limitations like less potent venom, less efficient, less economical, and highly laborious and time consuming (Devi *et al.,* 2016). After 1960's, some remarkable developments have be made in electrical method of bee venom extraction. In 1966, Gunnison used an electrical bee venom extractor associated with a cooling system to prevent the volatilisation of bee venom components. In 1981, Pence used another method to prevent the evaporation of volatile compounds and thus deployed the underwater collection procedure to collect most potent venom. Electrical bee venom extractions are conducted to learn the most effective method both in terms of quality and quantity by modifying the electricity source, voltage and frequency of electric shock, exposure period to electric shock and time interval between two electric shocks.

A number of factors affect the production quantity and quality of bee venom from honeybees. The amount of venom extraction varies according to age as venom content of newly emerged, 6 days old, 11 days old and 15 days old bees amounts to negligible, 0.05mg, 0.07mg and 0.10 mg dry weight respectively (Palmer, 1961). Normally 0.15 to 0.3 mg of venom held in a full venom sac of honeybee (Eze *et al.,* 2016). Other than age the factors affecting bee venom production quantitatively are the time of collection in a day, season of collection and on what position does the venom collecting board have been placed. The toxicity of *A. cerana* venom is twice as high as that of *A. mellifera*. *Apis* venom especially of *A. mellifera* is considered to be the best characterized venom in the Hymenoptera (Surendra *et. al.,* 2011). The venom collected by surgically removing the venom sac showed different protein content than that collected by electric shock method (Devi *et al.,* 2016).

Besides these above mentioned factors, release of alarm pheromone along with bee venom triggers various reactions among honeybees like stress and behavioural responses in bees, which affects the regular activities of bee hive. Various researches have been conducted to study the effect of bee venom extraction on behaviour and activities of honey bee colony by conducting experiments on honey bee responses. Honey bees show a distinctive response to electrical method of extraction, the learning effect. Bees try to avoid the electrical wire grid as

a habitual learning response. This constraint can be eliminated by keeping a period of gap between two successive extractions.

As harvesting of bee venom can provide a bee keeper an extra income even also in dearth period, extraction of bee venom has considerable importance. Because of the large market scope in the field of cosmetics and medicines, its production can enhance the socio-economic status of a beekeeper. On this context, quality and toxicity of bee venom have an important role to play. Although there is no significant quality parameters for bee venom but some factors affects its quality such as sunlight, moisture, oxidation, contaminants etc. (Bogdanov, 2016). Protecting from above mentioned conditions can improve the quality and storage period of bee venom. Honey bee venom is also used in curing joint pains, rheumatism, arthritis (Jae-Dong *et al*., 2005; Berman *et al.*, 2000; Ali, 2102), bee venom allergy etc. under the term Bee venom therapy. Bees are made to sting directly to human body like an acupuncture. Thus toxicity of sting becomes important to keep in mind. Studies have been shown that the median lethal toxicity of bee venom is 2.8 mg/kg of body weight (Schumacher et al., 1989).

In India very little research regarding the effect of bee venom extraction on colony behaviour (foraging and defensive behaviour) and performance (brood rearing activity, honey and pollen collection) has been done and especially no work has been done in agro climatic zones of Haryana. Considering bee venom as an important part in the growth of bee keeping and lack of data on the effects of extractions on bee colonies; the present study has been planned with the aim to study the seasonal variations in venom yield and effect of bee venom extraction on colony behavior and performance with the following objectives:;

Objectives of investigation

i. To record the seasonal variation of bee venom quantity collected through bee venom extractor
ii. To study the seasonal variation in honeybee behavior due to the effect of bee venom extraction
iii. To study the seasonal variation in honeybee colony performance as a result of bee venom extraction

CHAPTER-2

REVIEW OF LITERATURE

As discussed in previous chapter, a number of factors are responsible for affecting quality and quantity of bee venom collection by venom extractor. Besides that, another matter of focus is the stress causing factors that in correspondence with bee venom collection brings about changes in routine activity of bees in bee hive. The principal factor which is responsible for these behavioural changes is the alarm pheromone, which releases along with bee venom during bee venom collection. Thus, efforts have been made to collect some available literature pertaining to the seasonal variation in bee venom extraction and its effect on *Apis mellifera* L. colony performance:

Palmer (1961) had conducted an experiment by using electric shock method to extract bee venom. He used electrocuted steel wire grid over agar gel sheet on which bee venom deposited. On course of his experiment, no mortality was observed. He also had analysed the extraction effect on honey bees' regular activities, which resulted as normal like colonies without bee venom extraction.

Barker *et al*. (1963) reported after bee venom extraction from honey bee colonies, the workers bees observed to be more ravenous. This behaviour caused them to consume more amount of honey from the colonies. Except this no other adverse effects are observed due to bee venom extraction.

Morse and Benton (1963) had used nylon parchment taffeta as a cover stretched over the glass plate of the bee venom collector for deposition of bee venom. As a result of his experiment bees under this process became irritable and more defensive where as it was also observed that out of all the bees subjected for bee venom extraction 99% of the bees were survived.

Nobre (1990) reported a venom collecting device using 12 V electric shocks to trigger the venom release. Rubber covered electric cables were used to reduce mortality of bees during extraction. He collected bee venom and store it at 0^{o}c or at domestic freezer.

Schumacher *et al.* (1990) had compared the bee venom quantity, lethality and toxicity of Africanised honey bee (*A. mellifera scutellata*) and European honey bee, and they recorded European honey bees (147 µg) contain more venom than Africanized honey bees (94 µg).

Schumacher *et al.* (1994) had conducted an experiment to record the rate and quantity delivered by a honeybee sting through an anesthetized rabbit skin and a filter paper disk. They noticed that it took almost same time i.e. 20 seconds to deliver 90% of venom from the sting.

After that a negative correlation observed between residual venom in sting and time, but in case of filter paper disk it took 30 seconds for 140 µg (approximately 100%) delivery of bee venom.

Kaviani-Vahid *et al*. (1995) conducted an experiment for standardization of bee venom to control *Bacillus subtilis*. They collected bee venom using electrical stimulation method and collected a total of 9203 mg of dry honey bee venom during spring and summer of 1993 and 1994 with purity of 82.82 ± 8.8 15%.

Simics (1995) reported due to study effect of bee venom extraction on mortality of bees. He reported 68 bees died per colony on an average. He also concluded that there is no significant effect of collection on mortality of bees.

Skubida *et al*. (1995) compared the effects of different bee venom collection methods with respect to amount of venom collected, colony performance, wintering and general productivity of honey, pollen, beeswax. They concluded collection methods had no impact on colony performance such as strength, brood rearing but had negative impact on wintering and productivity. They also studied the best method of venom collection was using collecting board frames on upper body or super chamber.

Fakhim-Zadeh (1998) used a cage structured device reported by Fakimzadeh (1990) for collection of bee venom. A quantity of 0.21 g of bee venom was collected cumulatively from 8 colonies with an average of 0.026 g/colony. The use of this device minimized over excitation of bees.

Bahreini *et al*. (2000) had constructed a bee venom extraction device in Iran, of size 42 × 50 × 58 cm with electric wire grid inside it. The voltage of this alternative current was 21 volt with an electric impulse of 3 sec at an interval of 7 sec. They collected 838 mg of bee venom from a colony in the 6 month experiment. As a result of this study, no adverse effect was observed on honey production of the honeybee colonies.

Khattab *et al*. (2000) had studied the effects of bee venom extraction on honeybee colonies and the effect of different races (Manzala, Carniolan and Italian bees) on bee venom collection during the honey flow seasons of 1997-1999. Bee venom was collected using different bee venom extractors with voltage ranging from 3- 14 Volts for 30 minutes/day. The amounts of bee venom obtained in all the runs were 0.313, 0.240 and 0.210 g/colony. They found that bee venom extraction had decreased the brood rearing from 208 inch to 81.6 inch per colony and mean area of honey and pollen from 263.33 inch to 203 inch per colony where as increased the mortality of bees from 15 to 16.6 per colony. They also noticed an increase in

temperature and relative humidity in hive after bee venom collection. Among the collection methods, battery with 12 V 16 A was proven effective and there was no significant difference in bee venom collection quantity (0.273g, 0.266g and 0.323g) among different races (Manzala, Carniolan and Italian bees, respectively).

Funari *et al.* (2001) used Africanised queen bees (*Apis mellifera*), Italian hybrids (*A. mellifera liguistica*) and carniolan hybrid (*A. mellifera carnica*) for studying the differences in bee venom productivity among different hybrids. According to the results of the experiment, they found that the carniolan hybrids (0.147±0.024 mg) contain higher amount of bee venom than other two honey bee queen races, whereas Africanised honey bee queen releases more (0.073±0.012 mg) venom than the others, in spite of having the lowest amount of bee venom in reservoir (0.117±0.015 mg).

Khodairy and Omar (2003) studied the effect of bee venom collection in Egypt on certain attributes of honeybee colonies like bee population, brood area, stored pollen area, stored honey areas, yield of the colonies, foraging behavior and the variation in venom amount collected. They found significant differences in bee venom amounts collected at different periods of active season maximum bee venom collected in June in comparison with May and July. They observed positive correlation between all the colony attributes and bee venom collection from the respective colonies.

Zhou *et al.* (2003) studied that the effect of electrical bee venom extraction on royal jelly and honey production was negative. The amount of honey production fell off by 45.64% to 49.90% and royal jelly to 46.17%, significantly. There was no change either in royal jelly production in each cup or the honey consumption volume. The larval acceptance was also dropped down by 31.05%, very significantly. Changes in population of honey bee observed after 28 days and 35 days of continuous bee venom extraction with an interval of 3 days.

Gholamian *et al.* (2006) studied the effect of venom collection on some behavioural characteristics such as defensive behaviour, general behaviour, queen's stability, wax production and comb making, honey yield and comparing the efficiency of venom collection by means of the two venom collectors, one used out of the hive and another used inside the hive. They observed that the venom collection by the venom apparatus used out of the hive had significant differences on the defensive behaviour and wax production and general behaviour in comparison with the control. Both of them had no abnormal effect on survival and queen's stability, honey yield, in comparison with the control. The rates of venom collection with these apparatuses were low. Venom apparatus used out of the hive had much trouble for the user during venom collection but venom apparatus used inside the hive was comparatively simpler.

Duran *et al*. (2011) studied the effect of apitoxin harvest at 20 and 30 days interval on weight of the colonies, mortality of bees and amount of apitoxin collected using bee venom collector. They couldn't find any significant difference in above mentioned attributes due to different frequencies of collection. They concluded 30 days interval is best for bee venom collection to reduce the labour and disturbance to bees.

Sanad and Mohanny (2013) studied the effect of bee venom collection with a modified collecting device of 12V AC on the average of dead workers, and the scale of sealed brood, with references to the effect of the period of the day, months and seasons on the weights of collected bee venom. Data were recorded 4 times a day i.e 4-6 am, 9-11am, 1-3 pm, and 4-6 pm from the month of March to November 2012. They observed that the highest venom was obtained during 4-6 pm (0.166 g/day) followed by 4-6 am (0.118 g/day), 9-11am (0.099 g/day) and 1-3 pm (0.080 g/day). Among the months, August gave the highest weights of bee venom (0.185 g / day) and the lowest weights of bee venom given by March (0.031 g/day). Among the seasons summer season gave the highest amount of bee venom (0.161 g/day) followed by autumn (0.116 g/day) and spring (0.040 g/day). Furthermore, the period of 1-3 pm was considered to be the safest as it gave the lowest numbers of dead workers as a side effect of gathering process (26.74 worker / day) compared to 33.49 workers/day during 9-11 am, 49.32 workers/day during 4-6 pm and the highest, 51.24 workers/day during 4-6 am. This study also revealed June as the month with highest dead worker bees (55.0 workers/day) and November with the least (14.1 workers/day). As per the three seasons maximum dead workers were found during summer (50.3workers/day) then spring (40.9 workers/day) and autumn (31.7 workers/day). The least side effect of gathering process on the decreasing area of sealed brood in comparison to control was recorded during November (11.3 %) and highest during July (18.1%). This decrement of sealed brood area was highest during summer season (16.9%) followed by spring (15.8%) and autumn (13.8%). From all the findings it was concluded that 4-6pm during August was best for bee venom collection as summer months gave highest amounts of bee venom and 1-3 pm was the safest period of collection due to minimum number of dead workers.

Ghazala *et al*. (2014) studied the effects of bee venom collection on honey and royal jelly yield of honeybee colonies by comparing colonies with bee venom collection and without bee venom collection, in spring 2013 in Egypt. Bee venom was collected by exposing the colonies to bee venom collector for 30 minutes. The bee venom quantity during the collection period of March, April and May were 0.498, 0.1176 and 0.1246 g/month respectively with an average of 0.246 g/month. Significant differences in production of honey and royal jelly between both these colony types were found and production of honey and

royal jelly was less in venom producing colonies (5.18 kg/ colony and 7.29 g/colony, respectively) than normal colonies (7.32 kg/colony and 9.38 g/colony, respectively).

Omar *et al.* (2014) were studied the effect of the artificial collection of bee venom with help of an electrical device of voltage 12 V during two years on the activity of honey bee colonies for the production of Royal jelly. Two local honey bee hybrids (open-mated queens) namely: Carniolan; *A. mellifera carnica* and Italian; *A. mellifera ligustica* were selected to be treated. In 2011, Carniolan hybrids gave bee venom on an average of 0.38 g/colony and Italian hybrid gave 0.33 g/colony. Descending order of bee venom production of F1 Italian hybrids were 0.4, 0.37 , 0.36 , 0.36 , 0.35 , 0.34 , 0.34, 0.3 , 0.3 gram / colony in August , July , May, October, September , June , March , April , February respectively whereas the order in F1 Carniolan hybrids were 0.47, 0.45, 0.44,0.42,0.38, 0.35, 0.34, 0.34, 0.3 g/colony in September, July, August, June, May, October, March, April, February, respectively. The descending order of bee venom amount produced in 2012 from F1 Italian hybrids were 0.40, 0.40 , ,0.40 , 0.37, 0.35, , 0.35,0.34 , 0.30 , and 0.30 g / colony in months May, July, August, September, April, June, March, February, and October, respectively while the order in F1 Carniolan hybrid were 0.45, 0.44 , 0.40, 0.38, 0.36, 0.35, 0.34 , 0.34, and 0.30 g / colony May, July, August, September , June , October , March, April ,and February respectively. They observed the highest royal jelly secretion for F1Italian hybrid 92.02, g / colony and for F1 Carniolan 92.8 g / colony in April while the lowest amount of royal jelly i.e. 56.51 g / colony for F1 Carniolan and 54.8 g/colony for F1 Italian hybrid in February. There was a decrease of 18.56 % ± 3.86 gm/ colony in the weight of the Royal jelly.

Hegazi *et al.*(2015) evaluated antimicrobial activity of bee venom produced by pure and hybrid race of Carniolan race of *A. mellifera* . They collected 46 ± 10.03 ml. gm. /colony from carniolan race and 75 ± 17.47 ml. gm. / colony from hybrid race. No significant difference in antibacterial activity of both type of bee venom were observed on these 5 pathogenic bacteria viz. *Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa*.

Modanesi *et al*. (2015) have investigated the period (morning and afternoon) and harvest time (30 min. and 60 min.) on apitoxin harvest by *A. mellifera* and its influence on defensive gene expression. This defensive gene expression is determined by swinging a black suede ball attached to a string, for a minute, in front of the entrance. Apitoxin harvest was done 3 times/week at morning 9 AM and afternoon 2 PM with 4 treatments (T1: morning/30 minutes, T2: morning/60 minutes; T3: afternoon/30 minutes; T4: afternoon/60 minutes). They observed that there was no difference in the time taken for first sting between treatments and control (1.3±0.7 to 1.4±0.8 seconds) and number of stings found on the black suede ball

(50.3±17.2 to 51.8±14.4). The highest amount of bee venom produced from T3 (49.1±13.0 mg/colony) followed by T1 (33.7±13.0 mg/colony), T4 (24.9±6.0 mg/colony) and T2 (24.6±10.0 mg/colony). They concluded that the better time for apitoxin harvest is in morning for 60 minutes with less stress to honeybee colonies.

Mohanny (2015) had studied the effect of different colours of glass plate (transparent glass- a, blue glass- b and mirror glass-c) used in bee venom collecting frame at three different positions of hive (top position-A, between the frames-B, bottom position- C) for venom production and mortality of bees. When frames were kept at top position amount of venom collected was on average 2.5776/ colony and dead bees were 1026.33/colony. In middle position when frames are kept between the hive frames venom amount collected was 2.3462 g/colony and dead bees were 547.33 workers/colony. In bottom position, venom amount was 2.1452 g/colony and dead bees were 720.33 workers/ colony. According to the results of his experiment, c type glass frames had highest amount of bee venom (9.3990 g/colony) compared to a type (6.0858 g/colony) and b type (4.8877 g/colony), where as mortality was also high for c type frames (2029 workers/colony, 604 workers/colony and 972 workers/colony) and lowest in b types (479 workers/colony, 463 workers/colony and 431 workers/colony) in top, middle and bottom positions respectively..

El-Bassiony *et al*. (2016) studied effect of bee venom collection board position and collection time on bee venom amount collected at 15 days interval. He observed increase in bee venom production at top position during spring, summer, autumn and winter season compared to front and bottom positions. They also observed maximum bee venom produced in sunset period than noon and early period. They also observed maximum worker brood activity in summer (140.184 $inch^2$/colony) followed by spring (90.150 $inch^2$/colony) and winter (4.266 $inch^2$/colony).honey area and pollen storage area followed the same trend as worker brood activity being highest in summer season (150.229 $inch^2$/colony and 112.651 $inch^2$/colony respectively).

El-Saeady *et al*. (2016) studied effect of bee venom collecting on the hygienic and hoarding behaviour of *A. mellifera* colonies. Effect of queen right and queen less colonies on bee venom collection was also studied. Bee venom collection method increased hygienic behaviour (22%) of honey bee worker cells as on average 65.3/100 cells were cleaned before treatment and 84/100 cells after treatment. Meanwhile there was no significant difference in hoarding behaviour before and after treatment in four colonies. The queen less had negative effect on venom quantity but number of comb had positive effect.

Nowar (2016) had extracted bee venom from the honeybee colonies from July to October at 3 different periods (10-12 am, 3-5 pm and 7-9 pm) and studied its effect on worker

brood area and number of dead bees in Egypt. He observed maximum venom obtained during July (0.17 g/colony and 0.20 g/colony) followed by August (0.19 g/colony and 0.18 g/colony) , September (0.14 g/colony and 0.11 g/colony) and minimum in October (0.09 g/colony and 0.08 g/colony) in 2014 and 2015 respectively. Maximum venom obtained during 7-9 pm followed by 10-12 am and 3-5 pm. Minimum death observed during 3-5 pm (62 bees/colony) followed by 10-12 am (65 bees/colony) and 7-9 pm (70 bees/colony). As an effect of bee venom collection highest decrement in worker brood area had observed during July month. He also reported colonies fed with sugar syrup produced lowest amount of bee venom than colonies fed with pollen substitute.

Onari *et al.* (2016) tested ten colonies of African-derived *A. mellifera*, each containing six brood frames, by dividing them into control (without apitoxin harvest); and treatment (with a biweekly harvest of apitoxin); to investigate the influence of apitoxin harvest on population development and hygienic behavior of *A. mellifera*. Defensive behaviour of colonies were also tested and they found that control colonies took 3.12 ± 2.10 s to sting first and treatment colonies took 3.12 ± 3.04 s to sting. Also the sting numbers didn't vary significantly (for control- 32.87 ± 18.81 stings and for treatment- 36.12 ± 20.15 stings on suede ball). The researchers also observed that apitoxin harvest caused significant reduction in the uncapped brood area of the colonies during the months of April (34.22 ± 9.31 sq. cm.), May (78.22 ± 18.63 sq. cm.) and June (53.36 ± 12.72 sq. cm.), and capped brood area in July (23.74 ± 19.30 sq, cm) compared to control (60.93 ± 12.45 sq. cm., 122.75 ± 19.73 sq. cm., 70.94 ± 18.45 sq. cm. and 108.70 ± 20.56 sq. cm. respectively). The hygienic behavior of the colonies was not affected by apitoxin harvest as there was no significant difference in results of hygienic behavior test (%) between control and treatment colonies.

Abrantes *et al.* (2017) compared the qualities of apitoxin such as moisture content, protein protein analysis and cytotoxicity assay with *Artemia salina,* collected from two different parts of collector. Type 1 apitoxin collected from glass slabs at the entrance to the hive and type 2 apitoxin from rinsing the waste accumulated in the collection rods with distilled water. In protein analysis they found Type 1 apitoxin (77.8%) had higher protein content than Type 2 apitoxin (51.9%). In cytotoxicity assays, Type 1 apitoxin (LD_{50} - 71.5µg mL^{-1}) was higher toxic than Type 2 apitoxin (LD_{50}- 191.6µg mL^{-1}). They concluded that the region where apitoxin accumulates in the collector influences the product quality of apitoxin.

Bovi *et al.* (2017) examined the effects of apitoxin harvest on development of Hypopharyngeal gland development by evaluating number and areas of acini. The experiment was conducted using two treatments: T1, without apitoxin harvest and T2, with apitoxin harvested by an electric collector and data recorded from honeybees from each treatment at one month interval. They found that T2 showed less number of acini and smaller acinar area,

thus concluded apitoxin harvest negatively affected the hypopharyngeal glands structure, which ultimately affects the production of royal jelly.

Omar (2017) examined the effect of collecting bee venom from Carniolan and Italian hybrid colony on brood rearing activity of such colonies. He observed that two hybrids collected a mean of 0.37 mg/colony and 0.39 mg/colony in first year, and 0.36 mg/colony and 0.39 mg/colony of bee venom in second year. The highest amount of bee venom were collected from both hybrid colonies in August in 2012 (0.45 mg and 0.44 mg respectively) and in June in 2013 (0.40 mg and 0.45 mg respectively). The descending order of bee venom amount collected in 2012 was 0.44mg (August) > 0.43 mg(July) >0.42 mg(September) > 0.41 mg(June) > 0.39 mg(May) >0.37 mg (October) >0.35mg (April) > 0.32 mg (March) > 0.31 mg(February) and for 2013 was 0.43 mg (June) >0.42mg (July) >0.41 mg (August) >0.40 mg (October) >0.39 mg (May) > 0.37mg (September) >0.36mg (April) >0.33mg (March) >0.29mg (February). In the first season (2012) the sealed brood areas in the test colonies were 273.68 $inch^2$/month/colony and 281.69 $inch^2$/month/colonies compared to the control colonies i.e. 291.86 $inch^2$/month/colonies and 307.72 $inch^2$/month/colonies. In second season (2013) sealed brood areas in test colonies were 252.83 $inch^2$/month/colonies and 264.22 $inch^2$ /month/ colony whereas in controls sealed brood areas were 293.31 $inch^2$/month/colony and 295.08 $inch^2$/month/colony. After analysis he concluded that there is no effect of bee venom collection on brood rearing activity of colonies.

Maulana *et al*. (2018) had reported a bee venom collector using a pulse wave generator and electric cell. This produced 12 V electric shocks to the bees for their sting response. The capacity of this extractor was 7-8 hours per day using solar energy as electric energy.

Bucio and Martinez (2019) had collected bee venom 13 times from August 2016 to June 2017 from honeybee colonies using 12 V electric current. They observed an average 52.41 ± 13.83 mg/hive bee venom production. They also noticed hives infested with *Varroa* mite *(V. destructor*) produce less bee venom with minimum during January 19.23 mg/hive and maximum amount obtained from infestation free hives during May (68.18 mg/hive).

Hussein *et al*. (2019) studied the effect of Italian and Carniolan hybrids and geographical location of Nasr city and Motobes region of Cairo on the honey bee venom production during different seasons, from September, 2017 to August, 2018. In Nasr city they collected 89.1mg/colony and 80.7 mg/colony from both (Carniolan and Italian, respectively) the hybrids during spring season, which is the highest followed by 48.9 mg/colony and 39.8 mg/colony in summer season, and the lowest, 15.9 mg/colony and 14 mg/colony in Autumn season. In Motobes region same trend was observed to be followed in dry bee venom

collection from Carniolan and Italian hybrids with the highest amount in summer season (86.0 and 77.6 mg/colony) followed by spring season (84.8 and 72.3 mg/colony) and the lowest amount in autumn season (78.3 and 62.4 mg/colony). On an average, they recorded 51.3 mg/colony and 44.8 mg/colony dry bee venom in Nasr city, and 83.03 and 71.43 mg/colony in Motobes region from Carniolan and Italian hybrids respectively with no significant difference. The study also revealed that in case of Carniolan hybrids the highest (May- 99.9 mg/colony and June- 102.5 mg/colony) and least (October- 11.1 mg/colony and November- 60.2 mg/colony) bee venom production time, and in case of Italian hybrid highest (May- 91.7 mg/colony and June- 89.5 mg/colony) and least (October- 9.4 mg/colony and November- 50.9 mg/colony) in Nasr city and Motobes geographical condition, respectively.

Parcela et al. (2020) studied the effect of colony strength, position of collection and collection time on bee venom quantity extracted through electrical stimulation. They observed maximum venom amount collected from strong colonies (0.193 g) than medium strong colonies (0.164 g) and between medium strong and weak colonies (0.111 g). Higher amount of bee venom collected at on frames position (0.165 g) compared to on entrance position of the hive (0.148 g). The highest amount of bee venom collected at morning hours (0.173 g) followed by early evening (0.166 g) and afternoon hours (0.131 g).

CHAPTER-3

MATERIALS AND METHODS

The present investigation entitled **"Seasonal variation in bee venom extraction and its effect on *Apis mellifera* L. colony performance"** was conducted at Apiary, Department of Entomology, CCS Haryana Agricultural University, Hisar during winter, spring and summer season in the year 2020-2021. The city of Hisar falls under the Semi-tropical region in the North- western zone of India. The materials and methods adopted for conducting this present investigation has been described below:

3.1. Recording the seasonal variation of bee venom quantity collected through bee venom extractor

3.1.1. Experiment details

The investigation is carried out in the Apiary and Experimental laboratory of Department of Entomology, CCSHAU, Hisar in three seasons i.e. winter, spring and summer, with different bee strength (6 frames/colony and 10 frames/colony) and different durations of exposure to bee venom extractor (30 minutes, 45 minutes and 60 minutes). Each treatment is replicated 3 times. The treatment and replication details of the experiment are given in Table 1.

3.1.2. Honey bee colonies

For the present investigation, 18 experimental honeybee colonies and 6 control colonies of European honeybee (*A. mellifera* L.) for each experimental season with respective honey bee strength i.e. 10 honeybee colonies with strength of 6 bee frames per colony and another 10 honey bee colonies with strength of 10 bee frames per colony were taken. These honeybee colonies have been reared in Apiary of CCS Haryana Agricultural University, Hisar, by natural rearing of broods in bee hive. Naturally bred and disease, parasite or predator free colonies were selected for experiment. Honey bee frames are equalized in each colony to their respective bee frame strength.

3.1.3. Bee venom extractor

Bee venom from experimental honey bee colonies is collected using electrical bee venom extraction method. A bee venom extractor of model SB-BVC manufactured from DPS Tech Smart Pvt. Ltd., New Delhi has been procured for the investigation purpose. The bee venom extractor is of size 310 mm in length, 220 mm in width, and 50 mm in height and weighs 1.6 kg. This bee venom extractor is used to provide intermittent electric shock of 9V to the honey bees. This collector consists of a parallel electric wire grid which is a good conductor of electricity with a glass plate beneath it.

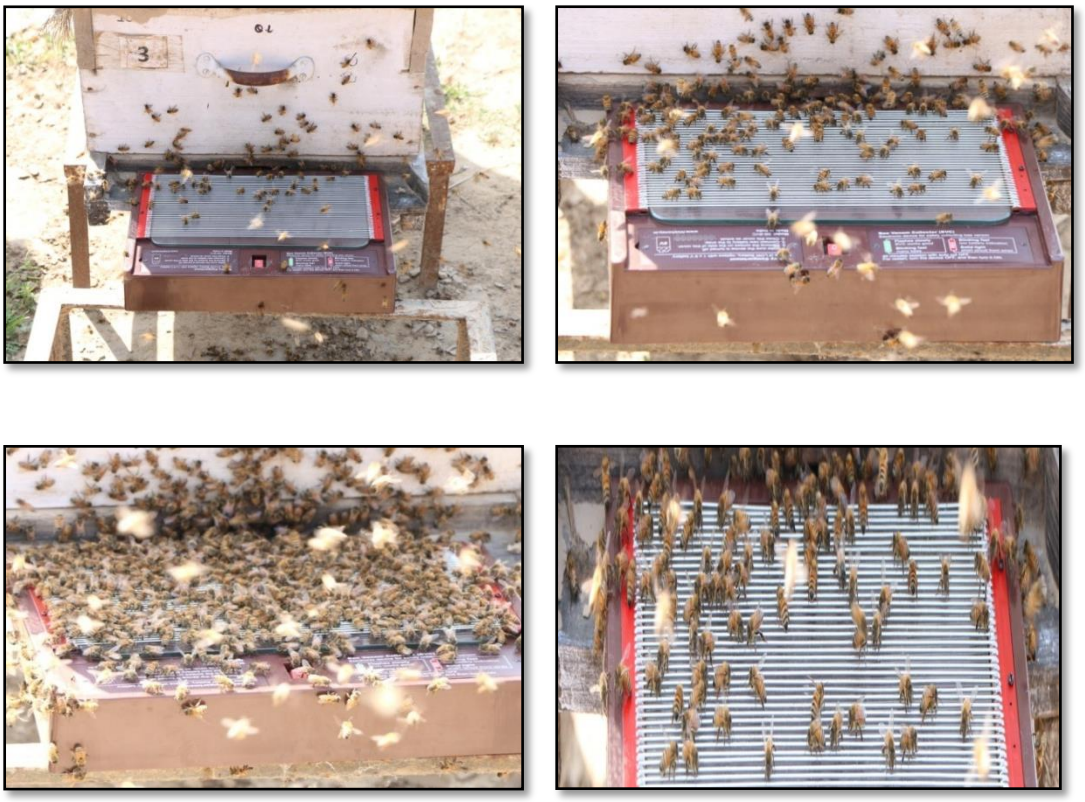

Plate 1: Bee venom extraction from the experimental honeybee colonies

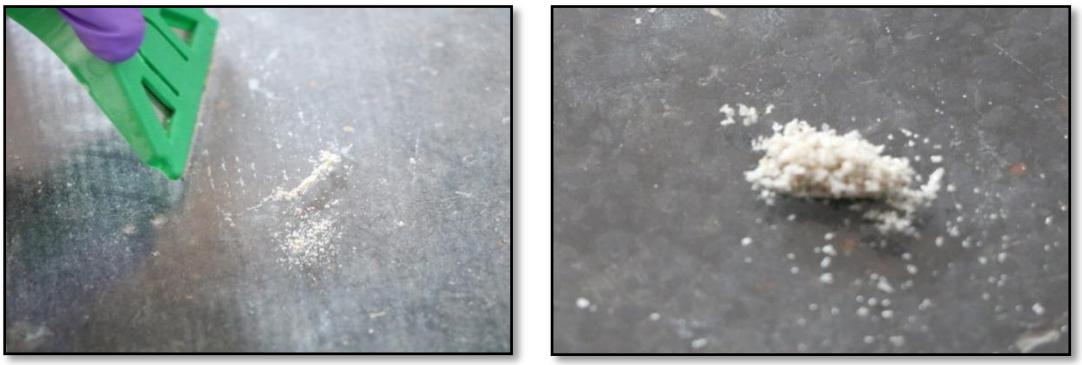

Plate 2: Collection of extracted bee venom from glass plate by scrapping

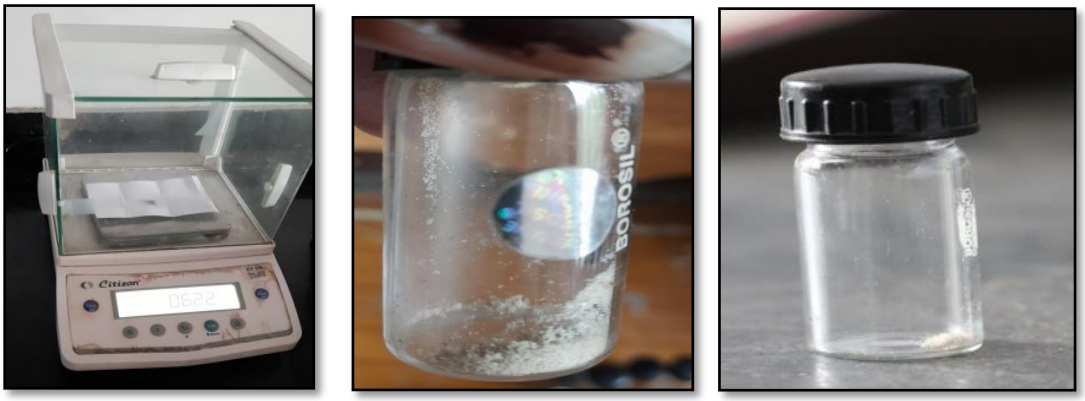

Plate 3: Weight measurement of bee venom with electronic balance and storage of bee venom

3.1.4. Methodology of bee venom extraction

This bee venom extraction apparatus is based on the principle of intermittent pulse oscillation and the characteristic biology of the honey bee workers. The extractor is placed at the entrance of experimental honey bee colony hives. When worker bees come in contact with this electrified wire grid, the electric shock of 9V makes the worker angry. This creates stress in worker bees and they ejaculate venom from its sting on the glass plate.

3.1.5. Observation recorded

The deposited bee venom on glass plate immediately dries out when comes in contact with air. The dried bee venom then scrapped out from the glass plate with a scrapper in the laboratory with following proper precautionary measures. The dried bee venom then weighed with an electronic balance. The quantity of bee venom extracted from each experimental colony from each season is recorded.

3.1.6. Storage of bee venom

The bee venom powder then stored in dark coloured glass bottle in deep freezer to protect it from temperature, moisture, sunlight and any oxidative substances. This storage method is known to improve the storage period to any length of time.

3.2. Seasonal variation in honeybee behavior due to the effect of bee venom extraction

The experimental colonies and treatment details is remained same for this objective of investigation. The observations on honey bee behaviour are recorded before the beginning of bee venom extraction in each season and again recorded the next day of bee venom extraction to find the difference in honey bee behaviour due to extraction effects.

3.2.1. Observations recorded

3.2.1.1. Time taken to regain normal defensive behavior by the colonies

Behavioural characteristics of the adult workers such as docile and high stinging are noticed for each colony before treatment. This behaviour is recorded using a black leather ball and jerking it at the entrance of bee colonies for 60 seconds before bee venom extraction as followed by Stort (1974), Delaplane and Harbo (1987), Brandeburgo and Gonçalves (1990) and Onari *et al.* (2016). The next day (after 24 hours of bee venom extraction) the defensive behaviour is recorded again. This observation is recorded with an interval of 24 hours until the colonies to regain its' normal behaviour. Data on the time taken are recorded after each venom extraction.

Plate 4: Recording of honeybee foraging behaviour of experimental colonies

Plate 5: Recording colony growth parameters using counting frame

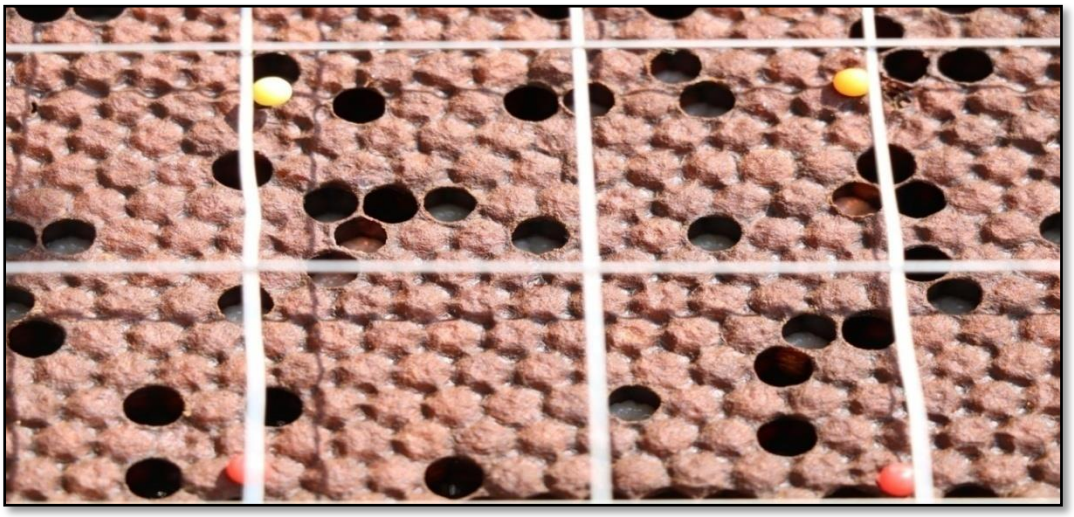

Plate 6: Recording brood survival rate of experimental honeybee colonies

3.2.1.2. Foraging behavior of bees

This behavioural characteristic is recorded next day after 24 hours of each treatment by taking following observations by similar method used by Eckert *et al*. (1994).

- Number of pollen foragers entering hive per 2 minutes are recorded by taking 10 observations. The observations are taken by counting the bees with pollen load on its hind legs.
- Number of nectar foragers entering hive per 2 minutes are recorded by counting the non-pollen foragers i.e. not having pollen load on hind legs, in 10 observations.
- Number of bees going out for foraging per 2 minutes and are recorded by counting the number of bees in 10 observations.

3.3. Seasonal variation in honeybee colony performance as a result of bee venom extraction

3.3.1. Methodology

The observations are recorded using a counting frame as used by Choudhury (2003). This counting frame was made up of an empty frame on with one inch square cells are made using a plastic wire. Thus the counting frame contains a total of 112 square inch cells arranging in 16 columns and 7 rows. The counting frame is placed over the normal frames of the experimental colony and the numbers of cells occupied by various parameters are conveniently recorded. The square inch of cells occupied with different parameters completely or with more than 50% part in the counting frame is considered as one.

3.3.2. Observations recorded

Colony performance is estimated by taking colony growth parameters into account. The growth parameters are recorded weekly after bee venom extraction from each treatment.

3.3.2.1. Worker brood rearing (square inches)

The numbers of cells containing pupae or capped brood with flat capping (worker brood) on all the frames in the colony were counted using the counting frame.

3.3.2.2. Drone brood rearing (square inches)

The numbers of cells containing drone brood (brood with convex capping and larger size) on all the frames in the colony were counted using the counting frame.

3.3.2.3. Brood survival

Brood survival rate was recorded by observing growth of egg stages of honeybees in areas of 25 sq. cm. marked with ordinary steel pins in beehive frames.

$$\text{Brood survival rate (\%)} = \frac{\text{Number of cells with hatched eggs/25 cm}^2}{\text{Number of cells with fresh eggs/25 cm}^2} \times 100$$

$$\text{Or} \quad \frac{\text{Number of cells with capped cells/25 cm}^2}{\text{Number of cells with larvae/25 cm}^2} \times 100$$

Growth of honeybee life stages were observed at weekly intervals. Based on the observation, brood survival rate was categorised into three groups;

- High brood survival rate: with 90-99% brood survival
- Medium brood survival rate: with 80-89% brood survival
- Low brood survival rate: with 70-79% brood survival

3.3.2.4. Mortality of bees

Mortality is recorded by counting the number of bees died due to the electric shock in each treatment, if any mortality occurred. The numbers of dead bees are counted by observing at the entrance of colony.

3.3.2.5. Honey storage (square inches)

Honey stored cells are present at outermost peripheral rows. The numbers of sealed or capped honey cells on all the frames in the colony were counted using the counting frame.

3.3.2.6. Pollen storage (square inches)

Pollen cells are present at the peripheral region internal to honey comb row. Pollen combs can be identified by noticing the cells packed with pollen. It appears in different shades of colours from bright orange and red to black. The numbers of cells containing pollen on all the frames in the colony are counted using the counting frame.

3.3.2.7. Fecundity

The numbers of cells containing eggs on all the frames in the colony were counted using the counting frame in square inches.

3.4. Statistical analysis

The data recorded under various subheads were tabulated and analysed using ANOVA for Randomised Block Design in OPSTAT software. The data on bee venom quantity extracted and mortalities observed were subjected to three factors ANOVA for RBD to know the effects of all three factors viz. frame strength of hives, period of exposure to bee venom extractor and seasons of extraction. Other parameters like behaviour and colony growth characters were analysed by subjecting the recorded data to two factors ANOVA for RBD to know the effects of frame strength and exposure period. The differences among the treatments were calculated at 5% level of significance.

Table 1: Details of Experiment

Season	Frequency of extraction / season	Treatment	Replication	Bee strength (no. of frames/colony)	Duration of exposure (in minutes)
Winter	2 (At 50th SMW* & 1st SMW)	T_1	3	6	30
		T_2	3	6	45
		T_3	3	6	60
		T_4	3	10	30
		T_5	3	10	45
		T_6	3	10	60
		Control-1	-	6	-
		Control-2	-	10	-
Spring	2 (At 8th SMW & 11th SMW)	T_1	3	6	30
		T_2	3	6	45
		T_3	3	6	60
		T_4	3	10	30
		T_5	3	10	45
		T_6	3	10	60
		Control-1	-	6	-
		Control-2	-	10	-
Summer	2 (At 17th SMW & 20th SMW)	T_1	3	6	30
		T_2	3	6	45
		T_3	3	6	60
		T_4	3	10	30
		T_5	3	10	45
		T_6	3	10	60
		Control-1	-	6	-
		Control-2	-	10	-

*SMW= Standard Meteorological Week

CHAPTER-4

EXPERIMENTAL RESULTS

The current investigation entitled **"Seasonal variation in bee venom extraction and its effect on *Apis mellifera* L. colony performance"** was undertaken during winter, spring and summer season of 2020-21 at Apiary, Department of Entomology, CCS Haryana Agricultural University, Hisar. The findings and experimental results of the investigation are presented in this following chapter under these subheadings below with help of tables and figures:

4.1. Seasonal variation of bee venom quantity collected through bee venom extractor

Bee venom production creates tremendous scope for a beekeeper by diversifying the source of income in both dearth and honey flow period. This makes the current investigation on seasonal variation in bee venom quantity so important.

4.1.1. Bee venom quantity extracted

Honey bee venom collected using the model SB- BVC apparatus from all the treatments in winter, spring and summer has been presented in table no. 2 and figure 1. As per the three factorial RBD analysis, bee venom amounts were significantly differed due to individual factors like frame strength viz. 6 frame hives and 10 frame hives; different durations of exposure to the bee venom extractor viz. 60 minutes, 45 minutes and 30 minutes; and seasons viz. winter, spring and summer. These bee venom quantities were not so differed significantly due to the inter-factorial effect. When compared between 6 and 10 frame strength hives, more amount of bee venom produced from 10 frame hives (0.016 g/ colony) than the 6 frame hives (0.009 g/colony) (Figure 5). From the results, it was clear that the colonies which are exposed to bee venom extractor for 60 minutes produce maximum quantity of bee venom (0.022 g/colony, 0.011 g/colony and 0.016 g/colony) followed by 45 minutes (0.015 g/colony, 0.009 g/colony and 0.012 g/colony) and 30 minutes (0.010 g/colony, 0.005 g/colony and 0.007 g/colony) from 10 frame hives, 6 frame hives and average of both, respectively (Figure 4). When compared within different treatments exercised during the investigation, the descending order of bee venom quantity produced/ > colony observed was; T_6 (0.022g/colony)> T_5(0.015g/colony)> T_3(0.011g/colony)>T_4 (0.010g/colony) T_2 (0.009g/colony) > T_1 (0.005g/colony) (Figure 2). Results stated bee venom amount extracted per colony varied from minimum 0.003 g in case of 6 frame colonies exposed to bee venom extractor for 30 minutes during 17th SMW of summer to maximum 0.052 g in case of 10 frame colonies

Table 2: Effect of different frame strength and period of exposure on quantity of bee venom extracted during different seasons of 2020-21

Seasons		Bee venom quantity (g)								
		6 frame hives				10 frame hives				Grand mean
		30 min	45 min	60 min	Mean	30 min	45 min	60 min	Mean	
		T_1	T_2	T_3		T_4	T_5	T_6		
Winter	50th SMW	0.008*	0.014	0.017	**0.013**	0.02	0.043	0.052	**0.038**	**0.025**
	1st SMW	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000	**0.000**	**0.000**
	Mean	**0.004**	**0.007**	**0.008**	**0.006**	**0.01**	**0.021**	**0.026**	**0.019**	**0.012**
Spring	8th SMW	0.011	0.016	0.017	**0.015**	0.015	0.02	0.037	**0.024**	**0.019**
	11th SMW	0.004	0.01	0.019	**0.011**	0.008	0.01	0.017	**0.012**	**0.011**
	Mean	**0.007**	**0.013**	**0.018**	**0.013**	**0.011**	**0.015**	**0.027**	**0.018**	**0.015**
Summer	17th SMW	0.003	0.006	0.005	**0.005**	0.007	0.006	0.013	**0.009**	**0.007**
	20th SMW	0.008	0.006	0.009	**0.007**	0.009	0.014	0.011	**0.011**	**0.009**
	Mean	**0.005**	**0.006**	**0.007**	**0.006**	**0.008**	**0.01**	**0.012**	**0.01**	**0.008**
Grand mean		**0.005**	**0.009**	**0.011**	**0.009**	**0.01**	**0.015**	**0.022**	**0.016**	**0.012**

*Each value is mean of 3 replications, SMW- Standard Meteorological Week

Factors	CD (p=0.05)	SE(m)
Frame strength	0.004	0.001
Duration of exposure	0.005	0.002
Interaction (frame strength x duration of exposure)	NS	0.003
Seasons	0.005	0.002
Interaction (frame strength x seasons)	NS	0.003
Interaction (duration of exposure x seasons)	NS	0.003
Interaction (frame strength x duration of exposure x seasons)	NS	0.004

exposed to bee venom extractor for 60 minutes during 50th SMW of winter. Among all three seasons, during spring maximum quantity of bee venom per colony (0.015 g/colony) has been produced followed by winter season (0.012 g/colony) and summer season (0.008 g/colony) (Figure 3).

4.2. Seasonal variation in honeybee behavior due to the effect of bee venom extraction

4.2.1. Time taken to regain normal defensive behavior

Observations for defensive behaviour was meant to be recorded by counting the number of stings on the black leather ball after shaking in front of the hive and comparing the numbers with the sting count of previous observation recorded before bee venom extraction. Although bees of the experimental colonies were coming in contact with the black leather ball, but they didn't sting on its surface. This response of the bees was due to lack of enough stimuli to trigger the stinging response in them probably. Thus the time taken for regaining the natural defensive behaviour couldn't be recorded without the bee sting count on the ball surface.

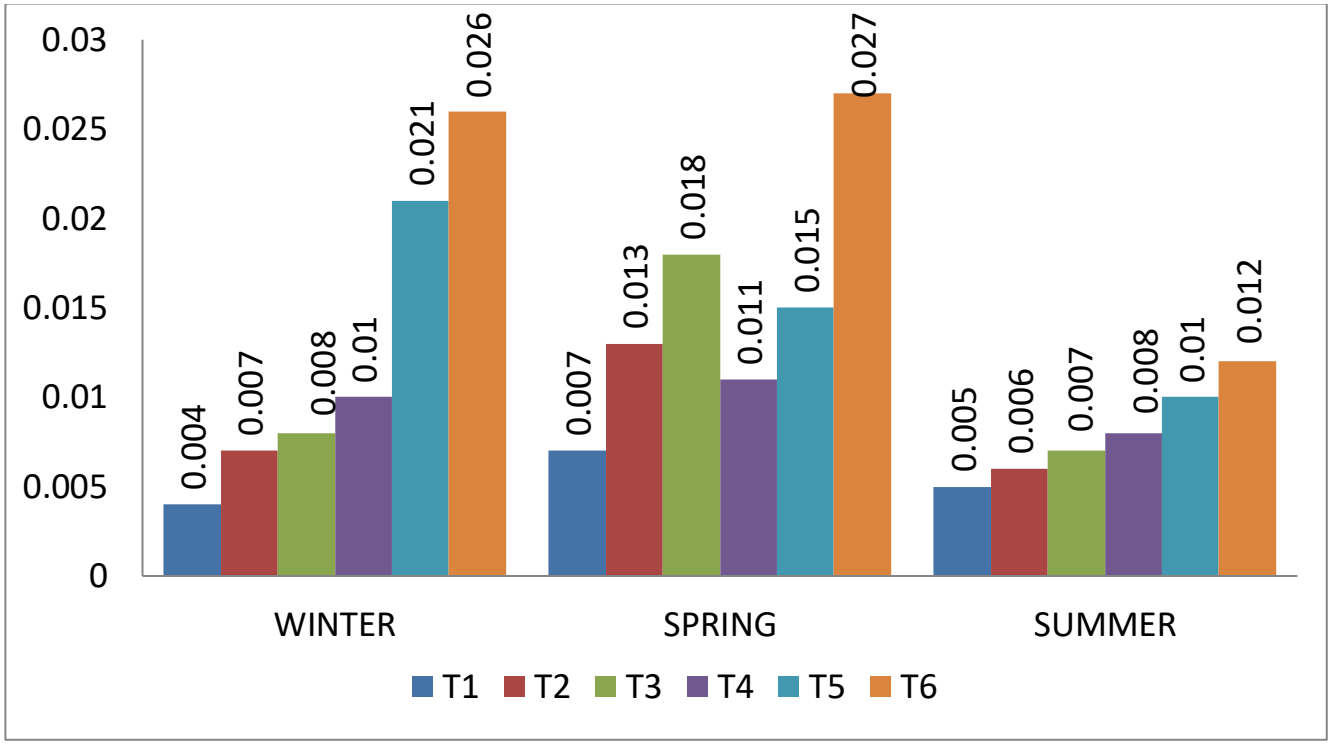

Figure 1: Bee venom amount collected from different treatments in different seasons during 2020-21

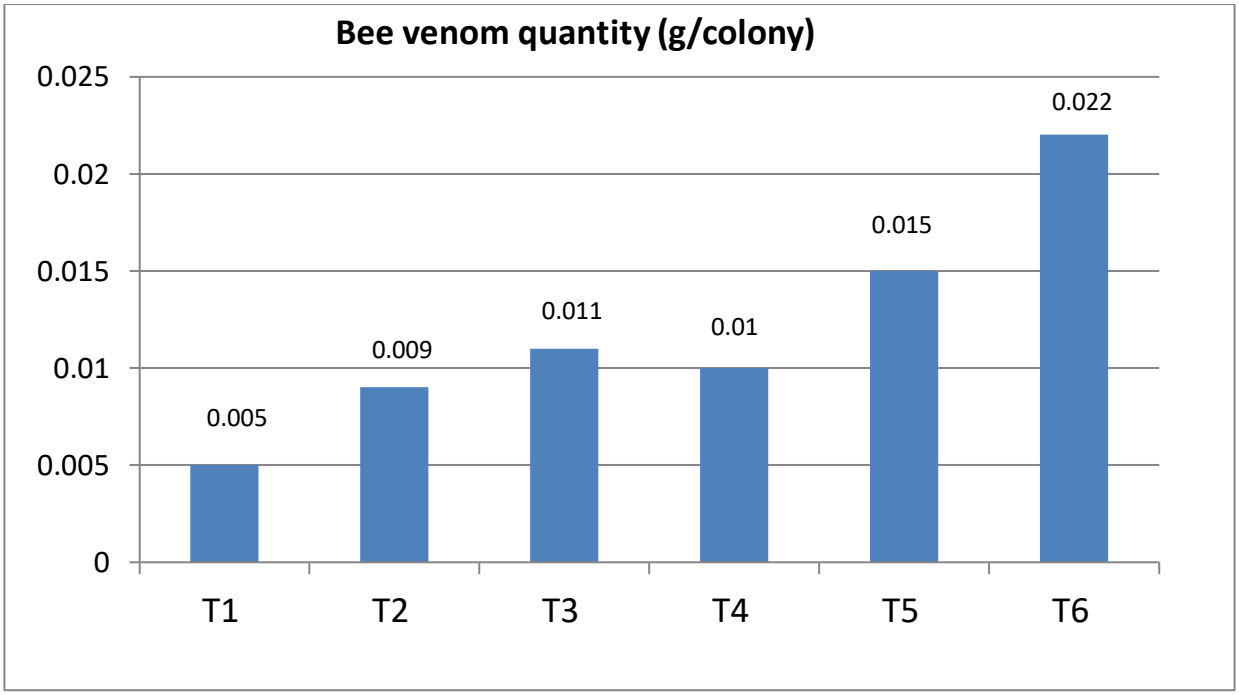

Figure 2: Effect of different treatments on bee venom quantity collected

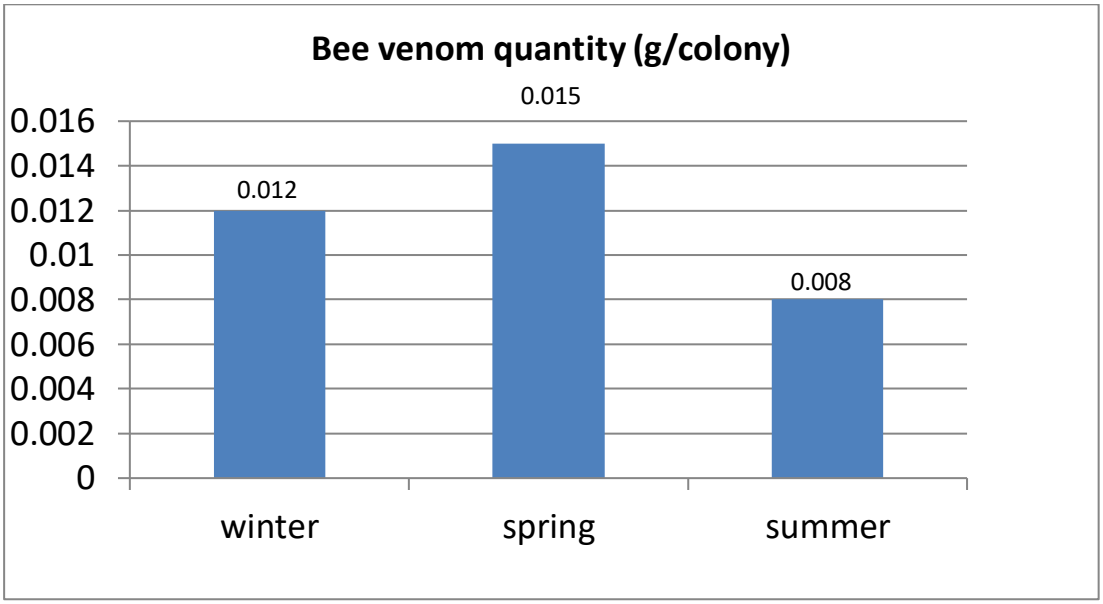

Figure 3: seasonal variation in bee venom quantity collected

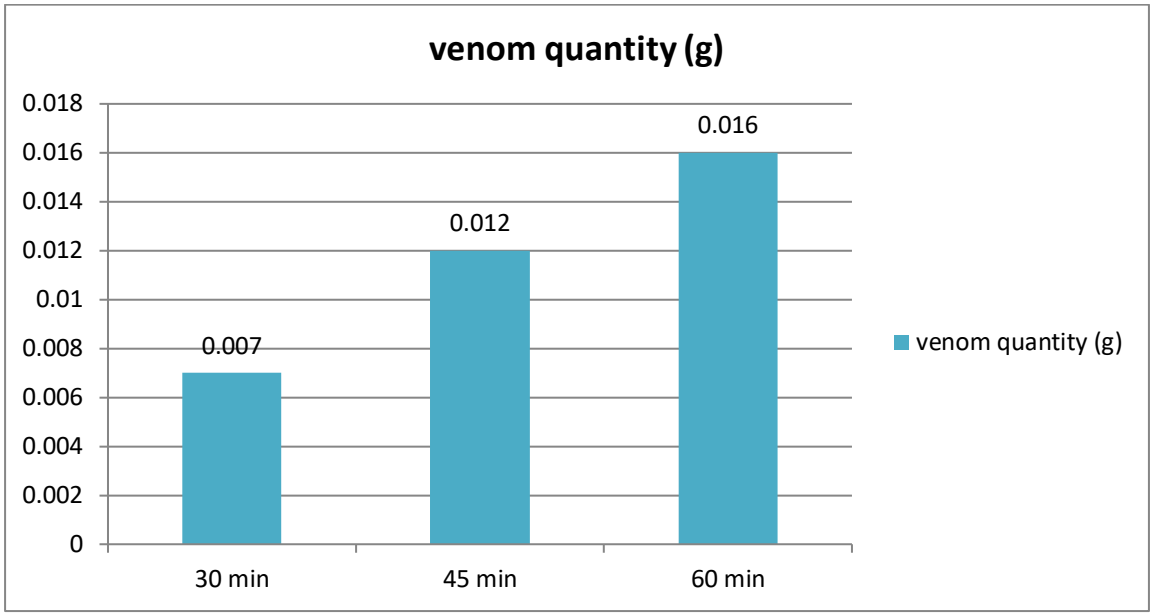

Figure 4: Effect of different period of exposure to bee venom extractor on bee venom quantity collected

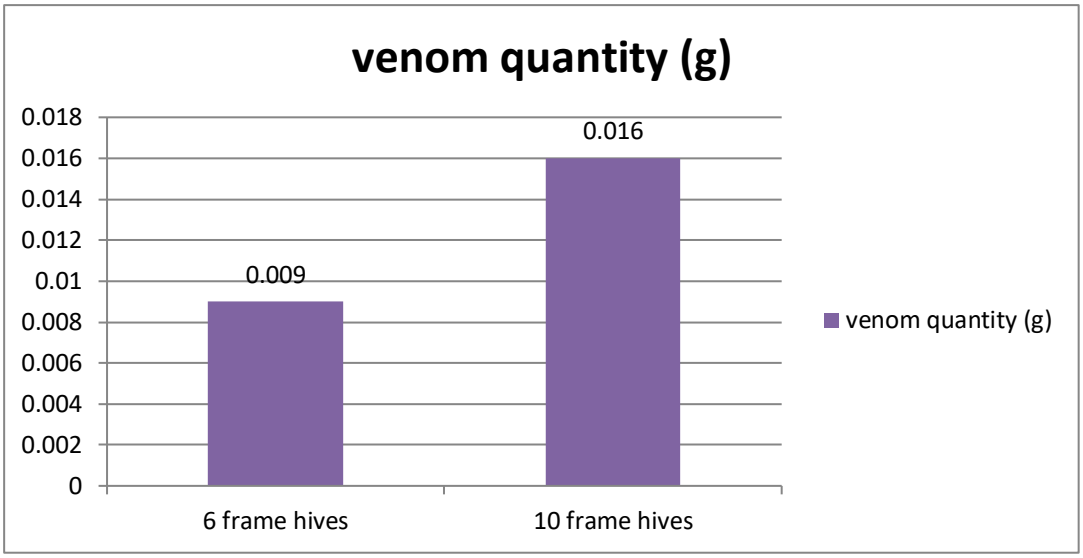

Figure 5: Effect of different frame strength on bee venom quantity collected

4.2.2. Foraging behavior

4.2.2.1. Pollen foragers entering the hive per 2 minutes

Data showed in table 3 indicates the effect of bee venom extraction on pollen foraging behaviour of experimental honey bee colonies by depicting the pollen foraging rate per hive per 2 minutes of both treated and untreated colonies. After the two factorial RBD analysis the results showed almost no significant difference among the numbers of pollen foraging bees entering the hive per 2 minutes due to frame strength of experimental hives and exposure period to bee venom extractor next day after the bee venom extraction except there was a significant difference observed during 11th SMW of spring season and 17th SMW of summer season due to the factor related to hive strength viz. 6 frame and 10 frame hives. The different durations of exposure of the honey bee colonies to bee venom extractor also had almost no effect on pollen forager population entering the hive except during the 17th SMW during initiation of summer. The interaction of both these above mentioned factors also had not significantly affected the pollen foraging activities of experimental honeybee hives. Thus it can be concluded that bee venom extraction or the electric shock produced by the bee venom extractor to collect bee venom form honey bees has no significant effect on pollen foraging activity of bees as period of exposure had not affected the colonies significantly.

Table 3: Effect of bee venom extraction on number of pollen foragers entering the hive per 2 minutes

Frame strength	Duration of exposure	Number of pollen foragers /hive min-2				
		WINTER	SPRING		SUMMER	
		50th SMW	8th SMW	11th SMW	17th SMW	20th SMW
6	**30 min**	17.73*	31.80	30.97	2.30	0.23
	45 min	17.00	35.60	27.80	3.00	0.37
	60 min	15.97	35.23	30.03	1.17	0.53
	0 min	19.93	35.27	27.83	2.60	0.67
	Mean	**17.66**	**34.47**	**29.16**	**2.27**	**0.45**
10	**30 min**	20.50	38.80	42.60	2.73	0.30
	45 min	21.57	37.47	38.67	5.23	0.30
	60 min	19.10	38.50	38.63	3.53	0.47
	0 min	21.73	40.37	41.53	7.70	0.77
	Mean	**20.73**	**38.78**	**40.36**	**4.80**	**0.46**

*Each data is mean of 10 observations, In winter extraction is done only once due to cessation of bee activity

SMW- Standard meteorological week

NS-Non-significant

Frame strength(A)	**CD (p=0.05)**	NS	NS	6.510	1.552	NS
	SE(m)	1.642	3.022	2.126	0.507	0.102
Duration of exposure(B)	**CD (p=0.05)**	NS	NS	NS	2.195	NS
	SE(m)	2.322	4.274	3.006	0.717	0.145
A X B	**CD (p=0.05)**	NS	NS	NS	NS	NS
	SE(m)	3.283	6.044	4.251	1.014	0.205

4.2.2.2. Nectar foragers entering the hive per 2 minutes

Table no. 4 depicted the nectar foraging behaviour of honey bees entering the experimental hives per 2 minutes on the next day of bee venom extraction from respective colonies. The mean nectar foraging rate of experimental colonies were analysed using two factorial RBD analysis and the results showed that there was almost no significant difference in nectar foraging activities of bees because of different hive frame strengths except during 11th SMW in spring season. There were no significant differences observed in nectar foraging rate of honey bees among the experimental colonies due to duration of exposure to bee venom extractor and due to interaction of both the pre-mentioned factors. According to the results, this can be taken as conclusion that there is no significant effect bee venom extraction on nectar foraging activities of honey bees of the colonies under investigation.

Table 4: Effect of bee venom extraction on number of nectar foragers entering the hive per 2 minutes

Frame strength	Duration of exposure	Number of nectar foragers /hive min-2				
		WINTER	SPRING		SUMMER	
		50th SMW	8th SMW	11th SMW	17th SMW	20th SMW
6	**30 min**	47.30*	30.63	36.57	43.70	38.67
	45 min	53.67	48.87	31.30	44.67	43.10
	60 min	43.77	33.17	34.63	51.00	34.87
	0 min	49.60	46.73	34.30	47.77	42.23
	Mean	**48.58**	**39.85**	**34.20**	**46.78**	**39.72**
10	**30 min**	50.77	34.13	50.57	53.80	35.57
	45 min	58.83	33.87	49.87	58.80	40.87
	60 min	57.97	49.57	40.60	59.83	34.43
	0 min	58.63	41.53	47.33	57.63	40.10
	Mean	**56.55**	**39.77**	**47.09**	**57.52**	**37.74**

*Each data is mean of 10 observations, In winter extraction is done only once due to cessation of bee activity

SMW- Standard meteorological week NS-Non-significant

Frame strength(A)	**CD (p=0.05)**	NS	NS	10.12	NS	NS
	SE(m)	3.885	3.758	3.304	3.612	1.796
Duration of exposure (B)	**CD (p=0.05)**	NS	NS	NS	NS	NS
	SE(m)	5.495	5.315	4.673	5.108	2.54
A X B	**CD (p=0.05)**	NS	NS	NS	NS	NS
	SE(m)	7.771	7.516	6.609	7.224	3.592

4.2.2.3. Number of bees going outside the hive per 2 minutes

In case of bees going outside from the experimental colonies for foraging activity were considered, the results on numbers of bees going outside the hives per 2 minutes were showed in table no. 5. The two factorial analysis showed that there is no significant difference observed in number of bee going out for foraging from the experimental colonies per 2 minutes due to frame strength, duration of exposure to bee venom extractor and the interaction of both the factors. This

clearly indicated that activity of bees going outside for foraging purpose is not significantly affected due to the process of bee venom extraction. Figure 6 represents foraging behaviour of the colonies in all three seasons with respect to different frame strength

Table 5: Effect of bee venom extraction on number of bees going outside the hive per 2 minutes

Frame strength	Duration of exposure	Number of bees going outside /hive min-2				
		WINTER	SPRING		SUMMER	
		50th SMW	8th SMW	11th SMW	17th SMW	20th SMW
6	30 min	54.10*	74.13	63.57	40.47	30.83
	45 min	54.07	69.57	55.73	42.80	39.43
	60 min	45.40	58.33	55.83	45.30	30.30
	0 min	52.87	67.80	58.80	43.60	34.20
	Mean	**51.61**	**67.46**	**58.48**	**43.04**	**33.69**
10	30 min	50.63	65.93	78.60	47.57	27.27
	45 min	61.83	65.30	63.17	51.90	33.00
	60 min	55.77	71.67	64.87	54.17	27.10
	0 min	58.60	68.30	68.47	54.13	32.60
	Mean	56.71	67.80	68.77	51.94	29.99
Frame strength(A)	**CD (p=0.05)**	NS	NS	NS	NS	NS
	SE(m)	2.748	3.519	3.926	3.514	1.621
Duration of exposure (B)	**CD (p=0.05)**	NS	NS	NS	NS	NS
	SE(m)	3.886	4.976	5.553	4.969	2.292
A X B	**CD (p=0.05)**	NS	NS	NS	NS	NS
	SE(m)	5.496	7.037	7.853	7.028	3.242

*Each data is mean of 10 observations, In winter extraction is done only once due to cessation of bee activity
SMW- Standard meteorological week
NS-Non-significant

4.3. Seasonal variation in honeybee colony performance as a result of bee venom extraction

4.3.1. Sealed worker brood rearing

The effect of bee venom extraction due to electric shock method on worker brood rearing of experimental colonies has been shown in table no. 6, 7 and 8 for winter, spring and summer season respectively. The effects of different frame strength on worker brood area are shown in figure 7. In winter season, significant difference was observed in worker brood area due to frame strength of hives during all 3 observations at 7, 14 and 21 DAE. Honeybee hives with 10 frame strength had more worker brood area (283.67 $inch^2$, 364.09 $inch^2$, 335.25 $inch^2$) than hives with 6 frame strength (137.05 $inch^2$, 213.67 $inch^2$, 233.42 $inch^2$) at 7, 14 and 21 DAE respectively. No significant difference was observed in worker brood rearing due to duration of exposure to bee venom extractor and interaction between frame strength and period of exposure. This signifies that worker brood rearing hadn't affected significantly due to extraction of bee venom during winter season of 20202-21.

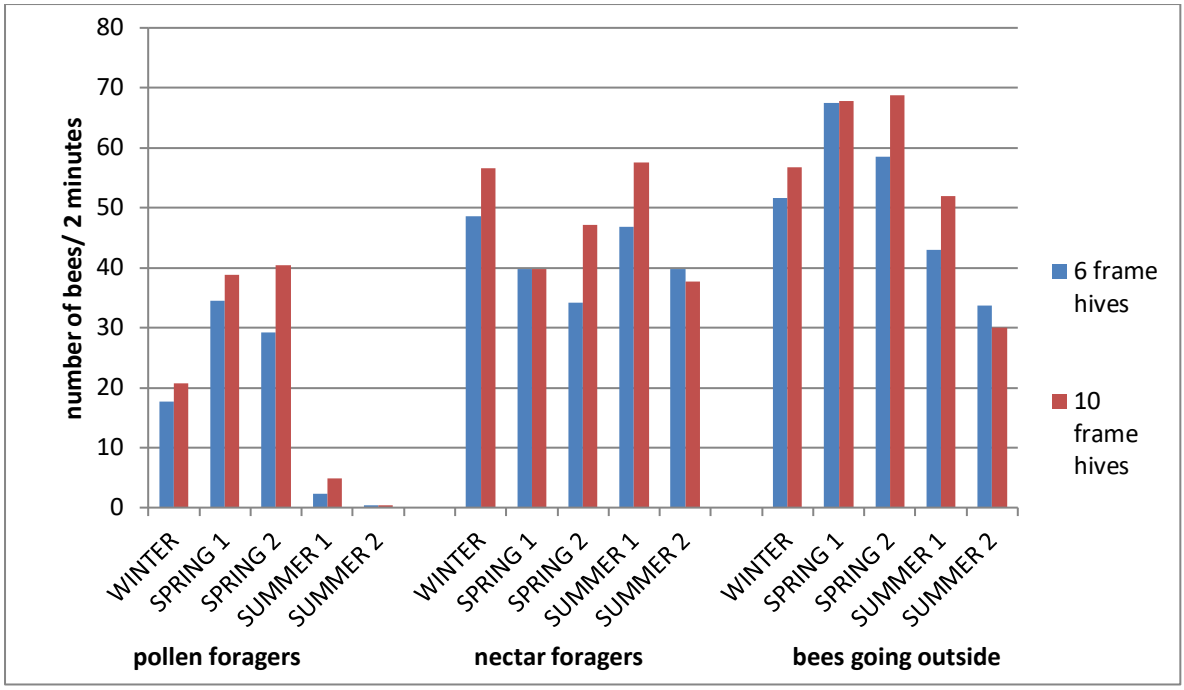

Figure 6: Effect of frame strength and different season on foraging behaviour of honey bee colonies during 2020-21

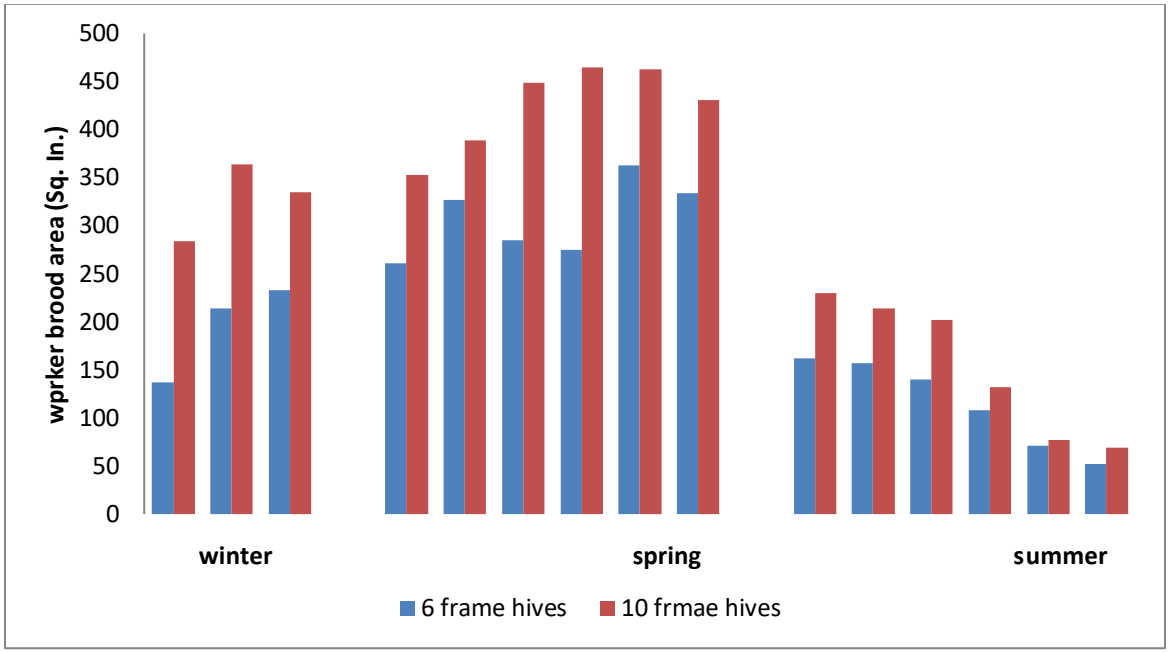

Figure 7: Effect of frame strength and different season on sealed worker brood rearing of honey bee colonies during 2020-21

In spring season, significant differences in worker brood rearing among experimental colonies were noticed due to frame strength of hives. Colonies with 10 frame strength had more worker brood area (viz. 352.84 inch^2, 389.25 inch^2, 448.67 inch^2, 465.09 inch^2, 462.17 inch^2, 431.08 inch^2) than 6 frame hives (260.42 inch^2, 327.08 inch^2, 285.34 inch^2, 274.83 inch^2, 363.09 inch^2, 334 inch^2) at 7, 14 and 21 days after first and second bee venom extraction respectively. Duration of exposure and interaction of it with frame strength had no significant effect on worker brood

area of experimental colonies, which indicates bee venom extraction process has no significant effect on worker brood rearing of colonies in spring season of 2020-21.

From table no. 8, it can be observed that significant difference due to different frame strength of hives was there among the colonies in case of worker brood rearing after first extraction of summer season. Same as winter and spring season, in summer season also 10 frame hives had more worker brood area (230 $inch^2$, 213.58 $inch^2$, 201.59 $inch^2$) than 6 frame hives (162.09 $inch^2$, 156.58 $inch^2$, 139.67 $inch^2$) at 7, 14 and 21 days after first extraction of summer season. No significant difference due to frame strength of colonies observed after second extraction of summer season. Duration of exposure to the bee venom extractor and its interaction with frame strength also hadn't posed any significant difference in worker brood rearing area after both the extractions of summer season. Thus it can be concluded that electric shock used for bee venom collection has no significant effect on worker brood rearing of experimental colonies during all three seasons of 2020-21.

Table 6: Effect of bee venom extraction on sealed worker brood rearing ($inch^2$) during winter season of 2020-21

Frame strength	Duration of exposure	Sealed worker brood area ($Inches^2$/colony)		
		7 DAE 14-12-2020	14 DAE 21-12-2020	21 DAE 28-12-2020
6	**30 min**	144.00*	239.67	185.00
	45 min	119.17	186.34	237.67
	60 min	124.34	213.67	267.00
	0 min	160.67	215.00	244.00
	Mean	**137.05**	**213.67**	**233.42**
10	**30 min**	225.34	324.34	296.34
	45 min	300.67	377.00	407.34
	60 min	284.00	374.67	299.67
	0 min	324.67	380.34	337.67
	Mean	**283.67**	**364.09**	**335.25**

*Each data is mean of 3 replications
In winter extraction is done only once due to cessation of bee activity
SMW- Standard meteorological week
DAE- days after extraction
NS-Non-significant

Frame strength (A)	**CD (p=0.05)**	43.909	68.189	73.427
	SE(m)	14.337	22.265	23.976
Duration of exposure (B)	**CD (p=0.05)**	NS	NS	NS
	SE(m)	20.276	31.488	33.907
A x B	**CD (p=0.05)**	NS	NS	NS
	SE(m)	28.674	44.531	47.951

Table 7: Effect of bee venom extraction on sealed worker brood rearing (inch^2) during spring season of 2020-21

Frame strength	Duration of exposure	Sealed worker brood area (Inches^2/colony)					
		Extraction -1			Extraction- 2		
		7 DAE 02-03-2021	14 DAE 09-03-2021	21 DAE 16-03-2021	7 DAE 24-03-2021	14 DAE 31-03-2021	21 DAE 07-04-2021
6	**30 min**	252.34*	280.34	249.67	293.67	419.34	328.67
	45 min	270.34	299.00	309.67	270.00	320.00	321.34
	60 min	247.67	357.00	293.34	255.00	306.34	346.00
	0 min	271.34	372.00	288.67	280.67	406.67	340.00
	Mean	**260.42**	**327.08**	**285.34**	**274.83**	**363.09**	**334.00**
10	**30 min**	329.00	344.67	470.67	446.34	481.67	425.00
	45 min	368.67	399.00	434.67	457.00	441.67	420.67
	60 min	336.34	415.34	428.00	467.67	457.67	437.00
	0 min	377.34	398.00	461.34	489.34	467.67	441.67
	Mean	**352.84**	**389.25**	**448.67**	**465.09**	**462.17**	**431.08**

*Each data is mean of 3 replications
SMW- Standard meteorological week
DAE- days after extraction
NS-Non-significant

Frame strength (A)	**CD (p=0.05)**	NS	NS	85.286	62.425	66.872	70.828
	SE(m)	40.367	43.445	27.848	20.383	21.835	23.127
Duration of exposure (B)	**CD (p=0.05)**	NS	NS	NS	NS	NS	NS
	SE(m)	57.088	61.441	39.383	28.826	30.879	32.706
A x B	**CD (p=0.05)**	NS	NS	NS	NS	NS	NS
	SE(m)	80.734	86.89	55.695	40.766	43.67	46.254

Table 8: Effect of bee venom extraction on sealed worker brood rearing (inch^2) during summer season of 2020-21

Frame strength	Duration of exposure	Sealed worker brood area (Inches^2/colony)					
		Extraction -1			Extraction- 2		
		7 DAE 04-05-2021	14 DAE 11-05-2021	21 DAE 18-05-2021	7 DAE 26-05-2021	14 DAE 01-06-2021	21 DAE 08-06-2021
6	**30 min**	180.34*	152.67	127.67	128.67	78.34	46.67
	45 min	167.00	149.00	150.34	84.34	64.67	62.34
	60 min	126.67	162.00	138.67	101.67	62.00	48.34
	0 min	174.34	162.67	142.00	116.00	78.00	53.00
	Mean	**162.09**	**156.58**	**139.67**	**107.67**	**70.75**	**52.59**
10	**30 min**	224.67	212.00	203.34	127.67	75.00	64.67
	45 min	220.00	212.00	199.34	130.34	92.00	78.67
	60 min	226.34	211.67	193.34	118.67	55.00	54.00
	0 min	249.00	218.67	210.34	152.34	85.67	77.67
	Mean	**230.00**	**213.58**	**201.59**	**132.25**	**76.92**	**68.75**

*Each data is mean of 3 replications
SMW- Standard meteorological week
DAE- days after extraction
NS-Non-significant

Frame strength(A)	**CD (p=0.05)**	39.487	31.647	33.839	NS	NS	NS
	SE(m)	12.893	10.333	11.049	11.149	11.087	9.457
Duration of exposure B)	**CD (p=0.05)**	NS	NS	NS	NS	NS	NS
	SE(m)	18.234	14.614	15.626	15.767	15.68	13.374
A X B	**CD (p=0.05)**	NS	NS	NS	NS	NS	NS
	SE(m)	25.787	20.667	22.099	22.298	22.174	18.914

4.3.2. Sealed drone brood rearing

Table no. 9 shows the effect of bee venom extraction on drone brood rearing area during winter. Results make it clear that no significant difference in drone brood rearing area was observed due to duration of exposure to the venom extractor but significant difference among colonies for drone brood area was noticed. The experimental colonies with 10 frames had more drone brood area (6.5 inch^2 and 22.63 inch^2) than 6 frame colonies (0.83 inch^2 and 9.33 inch^2) at 7 and 14 DAE respectively. During spring season also frame strength had significant on drone brood area but due to duration of exposure on there was no significant on drone brood rearing (Table 10). 10 frame hives had more done brood area (40.50 inch^2 and 48.92 inch^2) than 6 frame hives (13.92 inch^2 and 9 inch^2) at 14 and 21 days after first extraction of spring season. After second extraction of spring season, 10 frame hives had drone brood area of 34.83 inch^2, 43.09 inch^2 and 30.5 inch^2 at 7,14 and 21 DAE respectively, whereas 6 frame hives showed no drone brood rearing area.

Table 9: Effect of bee venom extraction on sealed drone brood rearing (inch^2) during winter season of 2020-21

Frame strength	Duration of exposure	Sealed drone brood area (Inches^2/colony)		
		7 DAE 14-12-2020	14 DAE 21-12-2020	21 DAE 28-12-2020
6	**30 min**	0.67*	8.67	12.00
	45 min	0.67	11.00	14.83
	60 min	1.00	7.33	12.33
	0 min	1.00	10.33	15.34
	Mean	**0.83**	**9.33**	**13.63**
10	**30 min**	6.67	21.67	30.00
	45 min	5.00	15.17	24.34
	60 min	6.67	27.33	27.00
	0 min	7.67	26.34	33.34
	Mean	**6.50**	**22.63**	**28.67**

*Each data is mean of 3 replications
In winter extraction is done only once due to cessation of bee activity
SMW- Standard meteorological week
DAE- days after extraction
NS-Non-significant

Frame strength(A)	**CD (p=0.05)**	2.961	10.754	NS
	SE(m)	0.967	3.511	5.181
Duration of exposure(B)	**CD (p=0.05)**	NS	NS	NS
	SE(m)	1.367	4.966	7.327
A X B	**CD (p=0.05)**	NS	NS	NS
	SE(m)	1.934	7.023	10.362

Table 10: Effect of bee venom extraction on sealed drone brood rearing (inch^2) during spring season of 2020-21

Frame strength	Duration of exposure	Sealed drone brood area (Inches^2/colony)					
		Extraction -1			Extraction- 2		
		7 DAE 02-03-2021	14 DAE 09-03-2021	21 DAE 16-03-2021	7 DAE 24-03-2021	14 DAE 31-03-2021	21 DAE 07-04-2021
6	**30 min**	22.00*	24.67	15.67	0.00	0.00	0.00
	45 min	10.00	6.33	3.00	0.00	0.00	0.00
	60 min	23.67	4.00	7.34	0.00	0.00	0.00
	0 min	24.67	20.67	10.00	0.00	0.00	0.00
	Mean	**20.08**	**13.92**	**9.00**	**0.00**	**0.00**	**0.00**
10	**30 min**	33.00	53.67	53.34	35.67	48.34	37.00
	45 min	33.33	32.34	38.67	30.00	43.00	14.00
	60 min	27.00	35.34	51.00	35.00	34.67	32.67
	0 min	31.67	40.67	52.67	38.67	46.34	38.34
	Mean	**31.25**	**40.50**	**48.92**	**34.83**	**43.09**	**30.50**

*Each data is mean of 3 replications
SMW- Standard meteorological week
DAE- days after extraction
NS-Non-significant

Frame strength (A)	**CD (p=0.05)**	NS	21.589	26.464	12.03	16.215	12.222
	SE(m)	7.632	7.049	8.641	3.928	5.295	3.991
Duration of exposure (B)	**CD (p=0.05)**	NS	NS	NS	NS	NS	NS
	SE(m)	10.793	9.969	12.22	5.555	7.488	5.644
A X B	**CD (p=0.05)**	NS	NS	NS	NS	NS	NS
	SE(m)	15.264	14.099	17.282	7.856	10.589	7.982

Table 11: Effect of bee venom extraction on sealed drone brood rearing (inch^2) during summer season of 2020-21

Frame strength	Duration of exposure	Sealed drone brood area (inch^2 /colony)					
		Extraction -1			Extraction- 2		
		7 DAE 04-05-2021	14 DAE 11-05-2021	21 DAE 18-05-2021	7 DAE 26-05-2021	14 DAE 01-06-2021	21 DAE 08-06-2021
6	**30 min**	0*	0	0	0	0	0
	45 min	0	0	0	0	0	0
	60 min	0	0	0	0	0	0
	0 min	0	0	0	0	0	0
	Mean	**0**	**0**	**0**	**0**	**0**	**0**
10	**30 min**	2	0	0	0	0	0
	45 min	1.67	0.67	0	0	0	0
	60 min	0.67	0.67	0	0	0	0
	0 min	1.67	0.84	0	0	0	0
	Mean	**1.50**	**0.54**	**0**	**0**	**0**	**0**
Frame strength (A)	**CD (p=0.05)**	0.679	0.441	NS	NS	NS	NS
	SE(m)	0.222	0.144	0	0	0	0
Duration of exposure (B)	**CD (p=0.05)**	NS	NS	NS	NS	NS	NS
	SE(m)	0.313	0.204	0	0	0	0
A X B	**CD (p=0.05)**	NS	NS	NS	NS	NS	NS
	SE(m)	0.443	0.288	0	0	0	0

*Each data is mean of 3 replications
SMW- Standard meteorological week
DAE- days after extraction
NS-Non-significant

During summer season significant difference observed at the beginning of season due to frame strength with 1.5 inch^2 and 0.54 inch^2 drone brood area in 10 frame hives at 7 and 14 days after first extraction and no drone brood area in 6 frame hives. At the later period no drone brood area recorded in any experimental colonies. As per the results showed in table 9,10 and 11; no significant difference due to exposure period implies there was no effect of venom extraction on drone brood rearing area of the colonies under investigation. Figure 8 shows the effect of frame strength on drone brood in all 3 seasons.

4.3.3. Brood survival

Effect of bee venom extraction on brood survival has been presented in table 12 during all three experimental seasons of 2020-21. Results showed high brood survival rate in almost all experimental colonies in all three seasons of 2020-21. There was no significant effect of frame strength, period of exposure and their interaction on brood survival rate of experimental colonies after each extraction, which signifies that the electric shock method used for bee venom extraction doesn't affect significantly the brood survival rate of the colonies.

Table 12: Effect of bee venom extraction on brood survival rate of experimental colonies during 2020-21

Frame strength	Duration of exposure	Winter	Spring		Summer	
6	**30 min**	97.92*	98.34	94.78	92.56	90.34
	45 min	96.56	94.23	96.34	91.67	90.56
	60 min	97.34	94.00	95.22	94.56	94.34
	0 min	97.78	93.34	96.67	92.67	92.00
	Mean	**97.40**	**94.98**	**95.75**	**92.86**	**91.81**
10	**30 min**	97.67	94.78	95.22	92.89	91.78
	45 min	95.56	96.22	96.11	94.56	89.22
	60 min	97.43	95.45	95.45	91.56	91.00
	0 min	98.00	95.45	96.34	94.34	92.45
	Mean	**91.16**	**95.47**	**95.78**	**93.34**	**91.11**

*Each data is mean of 5 observations
In winter extraction is done only once due to cessation of bee activity
SMW- Standard meteorological week
NS-Non-significant

Frame strength (A)	**CD (p=0.05)**	NS	NS	NS	NS	NS
	SE(m)	0.54	0.799	0.852	0.691	0.828
Duration of exposure (B)	**CD (p=0.05)**	NS	NS	NS	NS	NS
	SE(m)	0.764	1.129	1.205	0.977	1.171
A X B	**CD (p=0.05)**	NS	NS	NS	NS	NS
	SE(m)	1.08	1.597	1.705	1.382	1.656

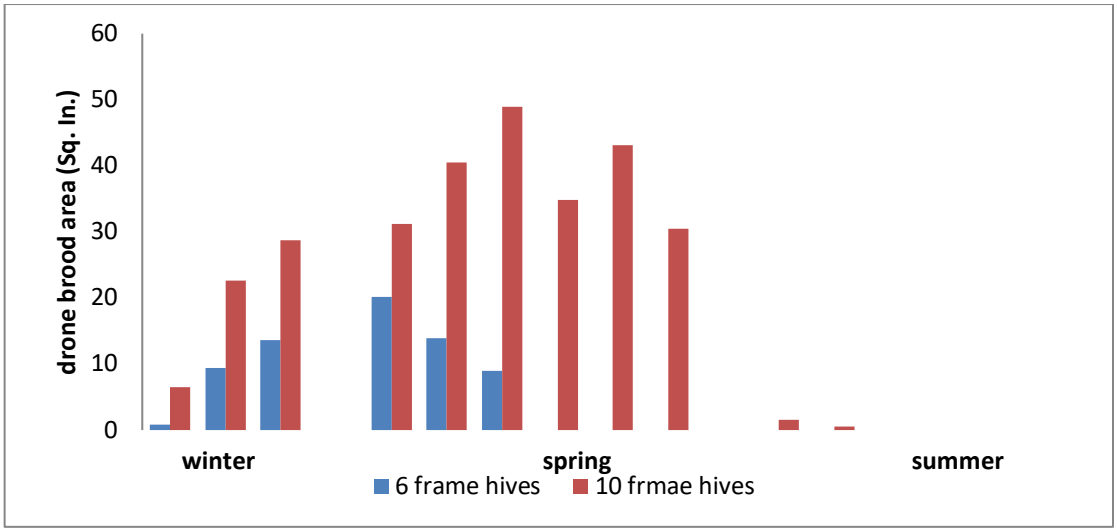

Figure 8: Effect of frame strength and different season on sealed drone brood rearing of honey bee colonies during 2020-21

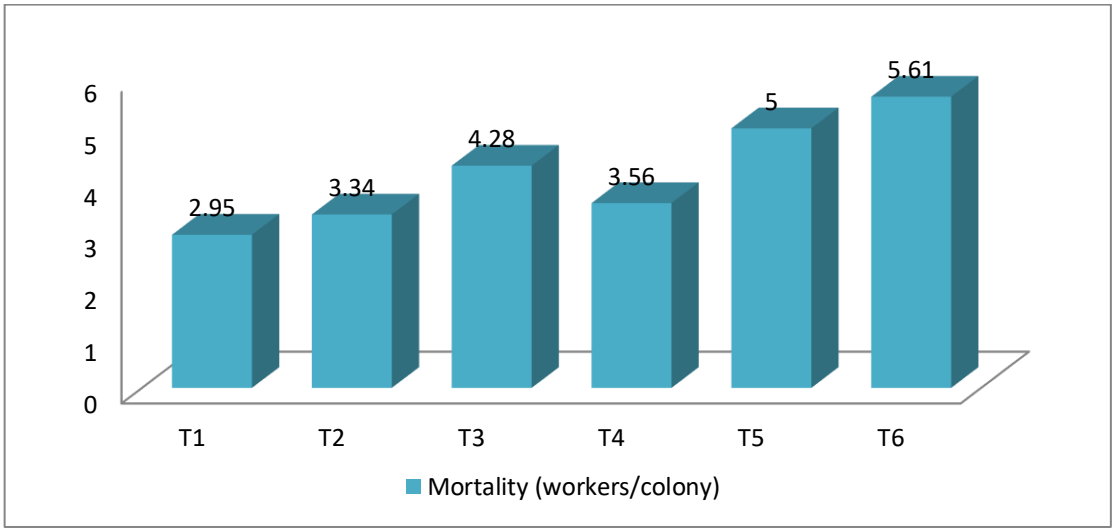

Figure 9: Effect of different treatments on bee mortality (workers/colony) of the colonies

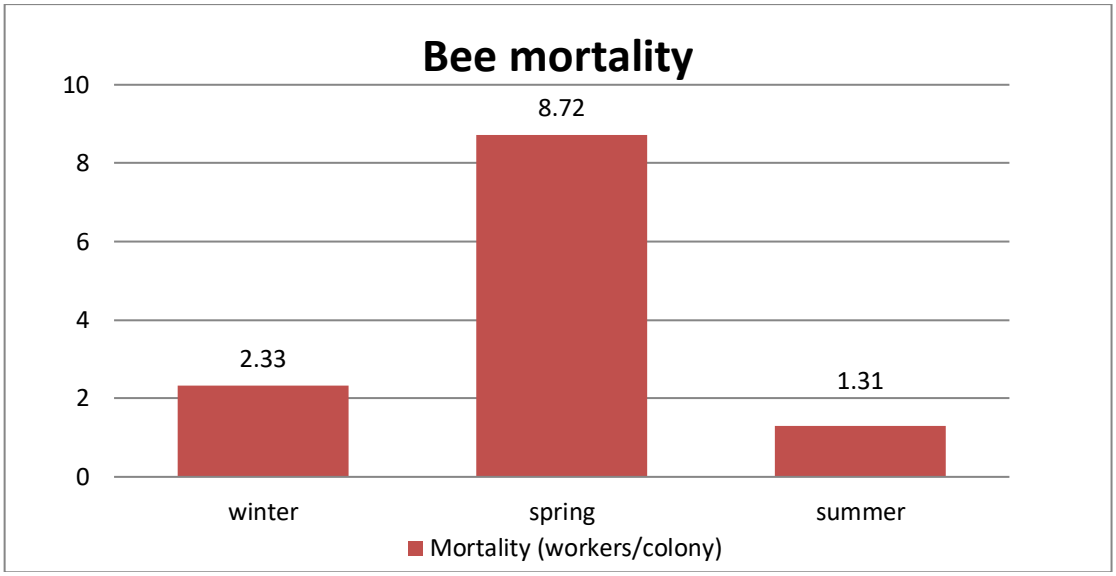

Figure 10: Effect of different seasons on mortality (workers/colony) of the colonies

4.3.4. Mortality of bees

The intermittent shock of 9V used for bee venom extraction imposed stress on honeybees to trigger defensive response in them, which resulted in mortality of some bees during the extraction. Table 13 presents the effect of frame strength, period of exposure and season on mortality of bees immediately after the extraction. As per the results of three factorial RBD analysis, the significant difference is observed due to all above mentioned individual factors but not because of their interaction. Hives with 10 frame strength showed more mortality of bees (4.72 workers/colony) compared to 6 frame hives (3.52 workers/colony) (Figure 12). Colonies exposed to bee venom extractor for 60 minutes showed maximum mortality of bees (5.61 workers/colony, 4.28 workers/colony and 4.95 workers/colony) followed by 45 minutes (5 workers/colony, 3.34 /colony and 4.17 workers/colony) and 30 minutes (3.56 workers/colony, 2.95 workers/colony and 3.25 workers/colony) with 10 frame strength, 6 frame strength and average of both, respectively (Figure 11). Among the treatments taken, the descending order of bee mortality was T_6 (5.61 workers/colony) >T_5 (5 workers/colony) >T_3 (4.28 workers/colony) >T_4 (3.56 workers/colony) >T_2 (3.34 workers/colony) >T_1 (2.95 workers/colony) (Figure 9). Spring season has recorded with maximum number of dead bees (8.72 workers/colony) followed by winter (2.33 workers/colony) and summer (1.31 workers/colony) (Figure 10). The above data indicates that the hives with lesser number of frames or with less bee strength experience lesser mortality, which stands same for duration of exposure to the bee venom extractor viz. for 30 minutes. Mortality of bees was directly proportional to the frame strength and period of exposure independently. Among the treatments T_1 was the safest for bee venom production in mortality point of view and among different seasons summer was the safest season of extraction.

For the most productive and safe bee venom extraction, bee venom quantities extracted were compared with their respective bee mortality shown in figure 13 -16. Although T_1 was recorded with minimum mortality, it was the colonies with the least amount of bee venom collected. In case of T_6, the gap between bee venom quantity collected and mortality of bees was widest (Figure 13). This makes T_6 the most productive and safe for bee venom extraction point of view. Similar conclusion has come up with the seasons. Although spring has recorded with maximum amount of bee venom, but it is also recorded with maximum number of dead bees. The gap between bee venom quantity and bee mortality is wider in winter season than other seasons of the experiments (Figure 14). Thus 10 frame hives with 60 minutes exposure to bee venom extractor during winter season is the best for both bee venom and mortality point of view (Figure 15 and 16).

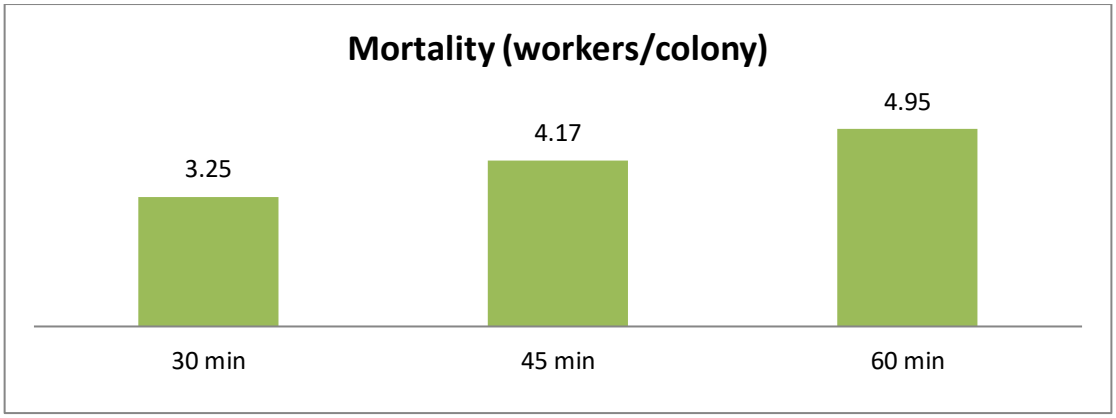

Figure 11: Effect of different periods of exposure to bee venom extractor on bee mortality (workers/colony) of the colonies

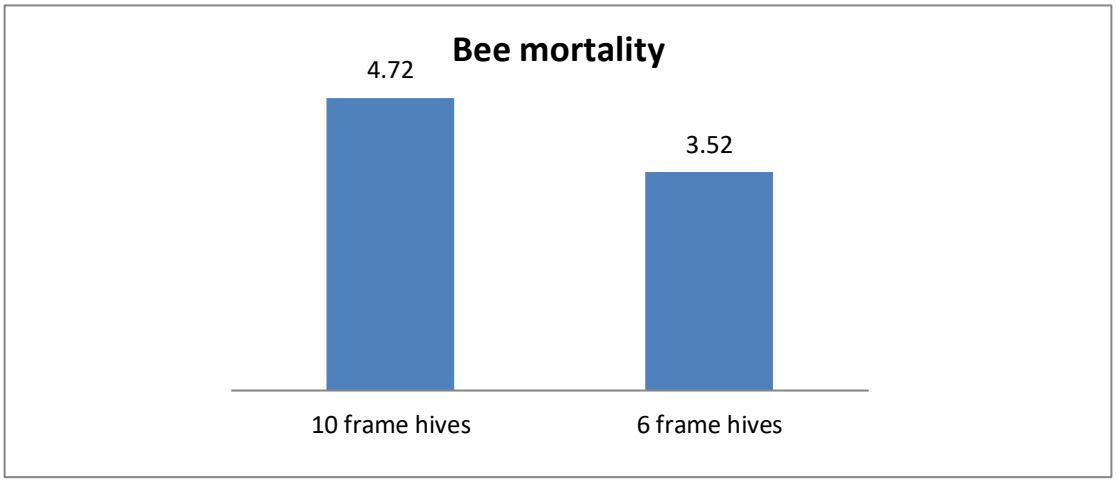

Figure 12: Effect of different bee frame strength on bee mortality (workers/colony) of the colonies

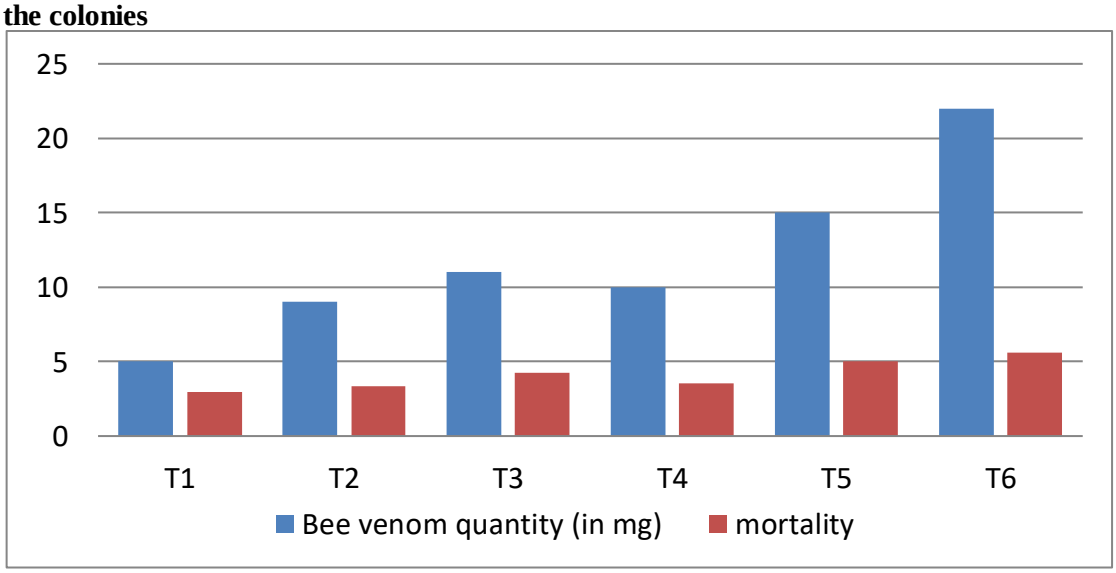

Figure 13: Effect of treatments on bee mortality (dead bees/ colony) in relation to bee venom quantity (mg)

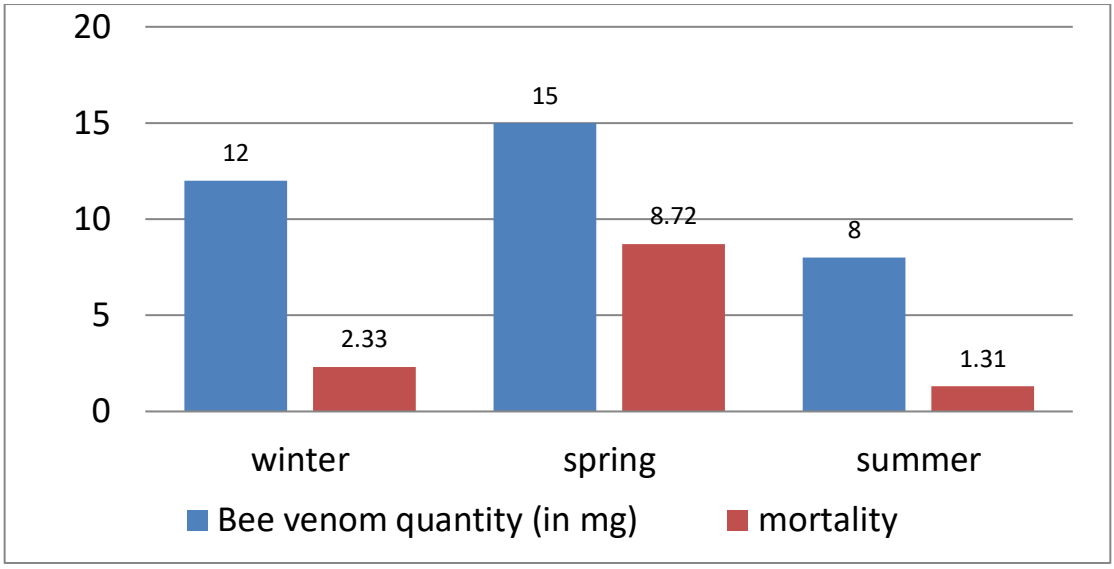

Figure 14: Effect of different seasons on bee mortality (workers/colony) in relation to bee venom quantity (mg)

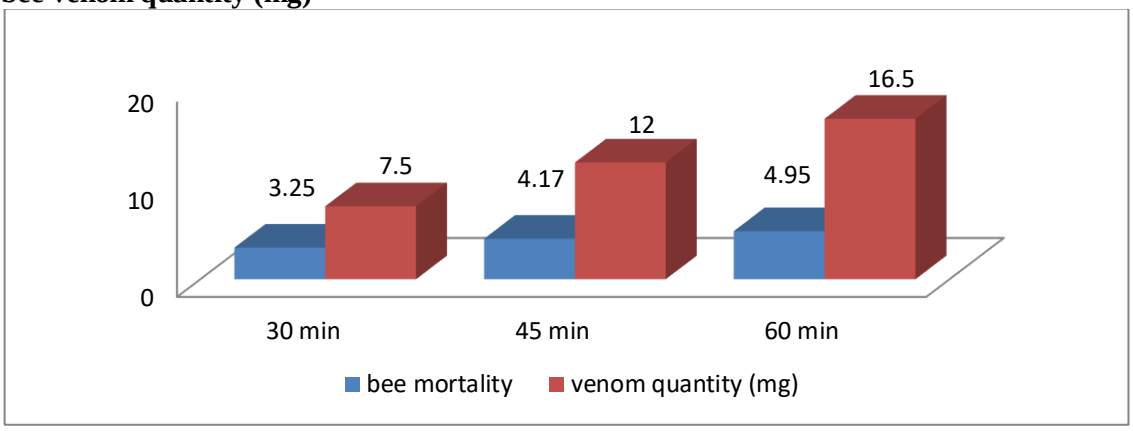

Figure 15: Effect of different duration of exposure on bee mortality (workers/colony) in relation to bee venom quantity (mg)

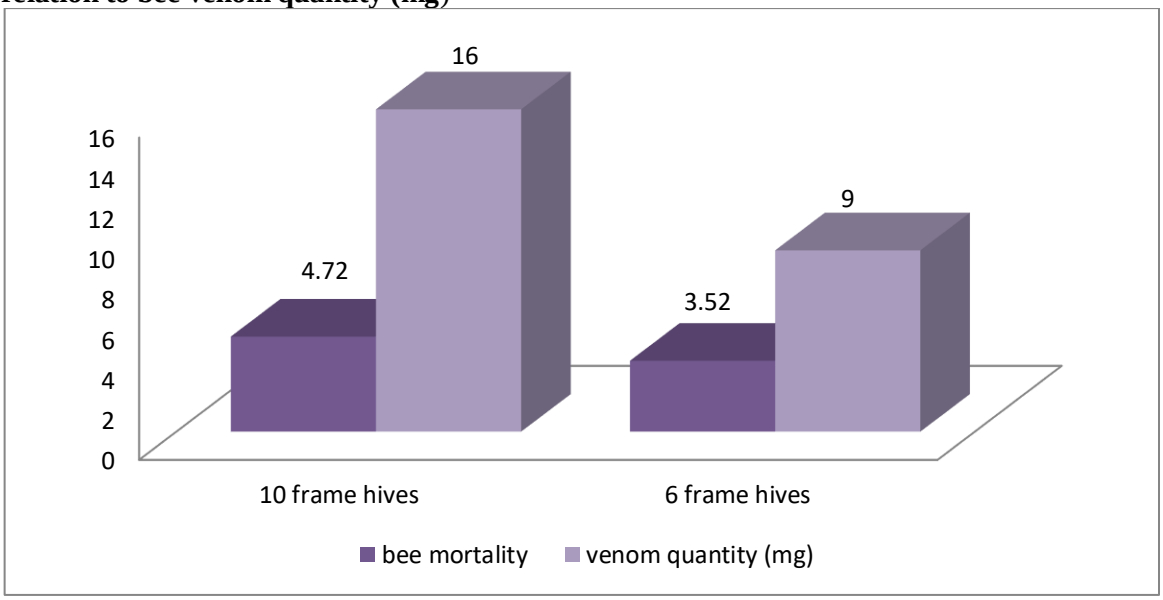

Figure 16: Effect of different bee frame strength on mortality of bees (workers/colony) in relation to bee venom quantity (mg)

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Table 13: Seasonal variation in effect of bee venom extraction on mortality of bees during 2020-21

SEASONS		Number of dead worker bees/ colony								
		6 frame hives				10 frame hives				GRAND MEAN
		30 min	45 min	60 min	MEAN	30 min	45 min	60 min	MEAN	
		T_1	T_2	T_3		T_4	T_5	T_6		
Winter	50th SMW	2.67*	3.00	5.30	**3.66**	3.67	6.00	7.34	**5.67**	**4.66**
	1st SMW	0	0	0	**0**	0	0	0	**0**	**0**
	Mean	**1.33**	**1.5**	**2.67**	**1.83**	**1.84**	**3.00**	**3.67**	**2.84**	**2.33**
Spring	8th SMW	7.67	9.67	12.00	**9.78**	9.67	15.33	18.67	**14.55**	**12.17**
	11th SMW	6.00	5.67	6.00	**5.89**	6.33	4.67	3.00	**4.66**	**5.28**
	Mean	**6.83**	**7.67**	**9.00**	**7.83**	**8.00**	**10.00**	**10.83**	**9.61**	**8.72**
Summer	17th SMW	0.70	0	1.33	**0.67**	0.67	1.00	2.67	**1.45**	**1.06**
	20th SMW	0.70	1.67	1.00	**1.12**	1.00	3.00	2.00	**2.00**	**1.56**
	MEAN	**0.70**	**0.84**	**1.16**	**0.90**	**0.83**	**2.00**	**2.34**	**1.72**	**1.31**
GRAND MEAN		**2.95**	**3.34**	**4.28**	**3.52**	**3.56**	**5.00**	**5.61**	**4.72**	**4.12**

*Each data is mean of 3 replications

SMW- Standard meteorological week, NS-Non-significant

Factors	CD (p=0.05)	SE(m)
Frame strength (A)	1.036	0.36
Duration of exposure(B)	1.269	0.441
A X B	N/A	0.624
Seasons (C)	1.269	0.441
A X C	N/A	0.624
B X C	N/A	0.765
A X B X C	N/A	1.081

4.3.5. Sealed honey storage

The effect of bee venom extraction on sealed honey storage of experimental colonies are presented in table 14, 15 and 16 for winter, spring and summer season of 2020-21 respectively. Significant differences have been observed during spring season due to frame strength with more sealed honey area in 10 frame hives (52.17 inch^2, 41.50 inch^2, 52.75 inch^2, 94.08inch^2, 179.08 inch^2, 115.42 inch^2) than 6 frame hives(16.83 inch^2, 11.16 inch^2, 19.92 inch^2, 22.75 inch^2, 37 inch^2, 39 inch^2) at 7, 14 and 21 days after first and second extractions, respectively. In case of summer season, significant difference due to frame strength is observed at 7 days after both the extractions in similar manner. 10 frame strength hives had more sealed honey area (104.25 inch^2 and 23.5 inch^2) than 6 frame hives (82.33 inch^2 and 16.42 inch^2) at 7 days after each extraction of summer season. There is no significant effect of exposure period on sealed honey area of the experimental colonies, which proves no relation between bee venom extraction and sealed honey storage. Figure 17 shows the effect of different frame strength on honey storage area of the colonies in all three seasons.

Table 14: Effect of bee venom extraction on sealed honey storage (inch^2) during winter season of 2020-21

Frame strength	Duration of exposure	Sealed honey storage (inch^2 /colony)		
		7 DAE 14-12-2020	14 DAE 21-12-2020	21 DAE 28-12-2020
6	**30 min**	5.34	39.00	48.00
	45 min	10.34	36.34	106.34
	60 min	11.00	46.34	164.67
	0 min	13.34	46.67	150.54
	Mean	**10.00**	**42.08**	**117.34**
10	**30 min**	5.34	48.67	204.34
	45 min	27.67	48.67	167.67
	60 min	18.00	48.34	145.00
	0 min	17.67	61.67	178.34
	Mean	**17.17**	**51.84**	**173.84**

*Each data is mean of 3 replications
In winter extraction is done only once due to cessation of bee activity
SMW- Standard meteorological week
DAE- days after extraction
NS-Non-significant

Frame strength(A)	**CD (p=0.05)**	NS	NS	NS
	SE(m)	4.538	8.349	22.314
Duration of exposure(B)	**CD (p=0.05)**	NS	NS	NS
	SE(m)	6.417	11.807	31.557
A X B	**CD (p=0.05)**	NS	NS	NS
	SE(m)	9.076	16.698	44.628

Table 15: Effect of bee venom extraction on sealed honey storage (inch^2) during spring season of 2020-21

Frame strength	Duration of exposure	Sealed honey storage (inch^2 /colony)					
		Extraction -1			Extraction- 2		
		7 DAE 02-03-2021	14 DAE 09-03-2021	21 DAE 16-03-2021	7 DAE 24-03-2021	14 DAE 31-03-2021	21 DAE 07-04-2021
6	**30 min**	8.00*	4.00	14.00	24.33	33.33	29.00
	45 min	13.00	9.67	19.00	11.67	12.33	33.67
	60 min	27.00	2.67	13.33	7.67	18.67	29.67
	0 min	19.33	28.33	33.33	47.33	83.67	63.67
	Mean	**16.83**	**11.16**	**19.92**	**22.75**	**37.00**	**39.00**
10	**30 min**	53.00	35.67	27.00	89.33	130.00	114.33
	45 min	23.00	21.67	10.67	55.67	191.33	123.00
	60 min	71.00	53.00	85.67	120.00	192.67	98.00
	0 min	61.67	55.67	87.67	111.33	202.33	126.33
	Mean	**52.17**	**41.50**	**52.75**	**94.08**	**179.08**	**115.42**
Frame strength(A)	**CD (p=0.05)**	18.408	NS	26.398	41.258	111.838	52.567
	SE(m)	6.011	10.087	8.62	13.472	36.518	17.164
Duration of exposure(B)	**CD (p=0.05)**	NS	NS	NS	NS	NS	NS
	SE(m)	8.5	14.265	12.19	19.052	51.644	24.274
A X B	**CD (p=0.05)**	NS	NS	NS	NS	NS	NS
	SE(m)	12.021	20.173	17.239	26.943	73.035	34.329

*Each data is mean of 3 replications
SMW- Standard meteorological week
DAE- days after extraction
NS-Non-significant

Table 16: Effect of bee venom extraction on sealed honey storage (inch^2) during summer season of 2020-21

Frame strength	Duration of exposure	Sealed honey storage (inch^2 /colony)					
		Extraction -1			Extraction- 2		
		7 DAE 04-05-2021	14 DAE 11-05-2021	21 DAE 18-05-2021	7 DAE 26-05-2021	14 DAE 01-06-2021	21 DAE 08-06-2021
6	**30 min**	94.00*	92.33	38.67	23.00	25.00	27.00
	45 min	63.33	47.00	30.67	10.67	14.67	31.67
	60 min	75.67	85.00	35.00	10.33	32.00	37.33
	0 min	96.33	76.00	41.33	21.67	32.00	44.00
	Mean	**82.33**	**75.08**	**36.42**	**16.42**	**25.92**	**35.00**
10	**30 min**	102.00	92.67	63.00	13.67	20.00	37.67
	45 min	87.33	64.00	34.00	15.67	18.67	26.67
	60 min	122.67	87.33	37.00	36.33	32.00	32.00
	0 min	105.00	86.00	47.67	28.33	31.33	46.33
	Mean	**104.25**	**82.50**	**45.42**	**23.50**	**25.50**	**35.67**

*Each data is mean of 3 replications
SMW- Standard meteorological week
DAE- days after extraction
NS-Non-significant

Frame strength(A)	**CD (p=0.05)**	18.349	NS	NS	6.345	NS	NS
	SE(m)	5.991	6.581	4.879	2.072	4.213	9.97
Duration of exposure(B)	**CD (p=0.05)**	NS	NS	NS	NS	NS	NS
	SE(m)	8.473	9.308	6.9	2.93	5.959	14.1
A X B	**CD (p=0.05)**	NS	NS	NS	12.69	NS	NS
	SE(m)	11.983	13.163	9.758	4.144	8.427	19.941

4.3.5 **Pollen storage**

Table 17, 18 and 19 show the relation between the treatments implemented on pollen storage of the colonies and bee venom extraction. Significant difference is noticed due to frame strength between 6 and 10 frame hives in winter, spring and initial period of summer. In winter at 7 and 14 days after extraction, it is seen that 10 frame hives (67.08 $inch^2$ and 94.67 $inch^2$) have more pollen area the 6 frame hives (35.25 $inch^2$ and 50.75 $inch^2$) respectively. During spring also 10 frame hives showed more pollen area (110 $inch^2$, 161.17 $inch^2$, 184.67 $inch^2$, 158.92 $inch^2$, 178.5 $inch^2$) than 6 frame hives (40.25 $inch^2$, 61.5 $inch^2$, 68.83 $inch^2$, 91.42 $inch^2$,110.25 $inch^2$) at 7, 14 and 21 days after first extraction and 7 and 21 days after second extraction respectively. In summer season at 7 days after first extraction 10 frame hives showed 159.17 $inch^2$ pollen area compared to 92.67 $inch^2$ in 6 frame hives. No significant difference in pollen storage area among the experimental colonies was observed due to duration of exposure to bee venom extractor. This signifies that there is no effect of bee venom extraction on pollen storage of honey bee colonies. Figure 18 shows the effect of different frame strength on pollen storage area of the colonies in all three seasons.

Table 17: Effect of bee venom extraction on pollen storage ($inch^2$) during winter season of 2020-21

Frame strength	Duration of exposure	Pollen storage area ($inch^2$/colony)		
		7 DAE 14-12-2020	14 DAE 21-12-2020	21 DAE 28-12-2020
6	**30 min**	36.00*	45.33	75.33
	45 min	38.33	52.33	83.33
	60 min	29.67	47.67	117.00
	0 min	37.00	57.67	146.00
	Mean	**35.25**	**50.75**	**105.42**
10	**30 min**	65.67	83.33	95.00
	45 min	60.67	88.67	86.33
	60 min	61.33	109.67	94.33
	0 min	80.67	97.00	99.33
	Mean	**67.08**	**94.67**	**93.75**

*Each data is mean of 3 replications
In winter extraction is done only once due to cessation of bee activity
SMW- Standard meteorological week,
DAE- days after extraction,
NS-Non-significant

Frame strength(A)	**CD (p=0.05)**	16.667	33.158	NS
	SE(m)	5.442	10.827	16.528
Duration of exposure(B)	**CD (p=0.05)**	NS	NS	NS
	SE(m)	7.696	15.311	23.374
A X B	**CD (p=0.05)**	NS	NS	NS
	SE(m)	10.884	21.653	33.055

Table 18: Effect of bee venom extraction on pollen storage (inch^2) during spring season of 2020-21

Frame strength	Duration of exposure	Pollen storage area (inch^2/colony)					
		Extraction -1			Extraction- 2		
		7 DAE 02-03-2021	14 DAE 09-03-2021	21 DAE 16-03-2021	7 DAE 24-03-2021	14 DAE 31-03-2021	21 DAE 07-04-2021
6	**30 min**	33.00*	67.67	60.67	82.00	130.00	98.00
	45 min	32.00	51.67	67.00	97.00	139.33	115.33
	60 min	36.33	55.00	62.33	86.00	120.67	101.33
	0 min	59.67	71.67	85.33	100.67	143.33	126.33
	Mean	**40.25**	**61.50**	**68.83**	**91.42**	**133.33**	**110.25**
10	**30 min**	115.67	162.67	169.67	160.33	179.33	174.67
	45 min	105.67	155.67	173.33	153.00	154.67	172.33
	60 min	107.67	164.67	173.00	158.67	144.33	176.67
	0 min	111.00	161.67	222.67	163.67	163.33	190.33
	Mean	**110.00**	**161.17**	**184.67**	**158.92**	**160.42**	**178.50**
Frame strength(A)	**CD (p=0.05)**	43.334	56.305	78.755	35.658	NS	38.92
	SE(m)	14.15	18.385	25.715	11.643	17.575	12.708
Duration of exposure(B)	**CD (p=0.05)**	NS	NS	NS	NS	NS	NS
	SE(m)	20.01	26	36.367	16.466	24.855	17.972
A X B	**CD (p=0.05)**	NS	NS	NS	NS	NS	NS
	SE(m)	28.299	36.77	51.43	23.286	35.15	25.416

*Each data is mean of 3 replications
SMW- Standard meteorological week
DAE- days after extraction
NS-Non-significant

Table 19: Effect of bee venom extraction on pollen storage (inch^2) during summer season of 2020-21

Frame strength	Duration of exposure	Pollen storage area (inch^2/colony)					
		Extraction -1			Extraction- 2		
		7 DAE 04-05-2021	14 DAE 11-05-2021	21 DAE 18-05-2021	7 DAE 26-05-2021	14 DAE 01-06-2021	21 DAE 08-06-2021
6	30 min	82.33*	84.67	60.33	25.33	17.67	17.00
	45 min	93.33	80.00	48.00	15.00	16.67	17.33
	60 min	86.33	81.00	40.00	30.33	21.00	14.67
	0 min	108.67	85.00	51.33	31.33	18.00	22.00
	Mean	**92.66**	**82.67**	**49.92**	**25.50**	**18.33**	**17.75**
10	30 min	192.67	131.00	52.00	31.33	24.00	19.67
	45 min	137.00	101.00	44.67	29.33	19.67	23.67
	60 min	147.00	96.00	51.00	34.00	20.00	18.33
	0 min	160.00	139.33	81.00	32.67	22.33	23.00
	Mean	**159.17**	**116.83**	**57.17**	**31.83**	**21.50**	**21.17**

*Each data is mean of 3 replications
SMW- Standard meteorological week
DAE- days after extraction
NS-Non-significant

Frame strength(A)	CD (p=0.05)	24.384	NS	NS	NS	NS	NS
	SE(m)	7.962	13.815	9.315	4.305	3.458	4.189
Duration of exposure(B)	CD (p=0.05)	NS	NS	NS	NS	NS	NS
	SE(m)	11.26	19.538	13.173	6.088	4.89	5.924
A X B	CD (p=0.05)	NS	NS	NS	NS	NS	NS
	SE(m)	15.924	27.63	18.629	8.609	6.915	8.378

4.3.6 **Fecundity**

The relationship between bee venom extraction and fecundity of honey bee colonies under investigation has been shown in table 20, 21 and 22 for winter, spring and summer season 0f 2020-21 respectively**.** Significant difference in fresh egg area between 6 and 10 frame hives are observed only during winter viz. 33.67 inch^2, 156.58 inch^2 and 196.42 inch^2 in 10 frame colonies compared to 20.67 inch^2, 42.33 inch^2 and 86.92 inch^2 respectively at 7, 14 and 21 days after extraction. No significant difference in area with egg brood was noticed due to frame strength in spring and summer season. Duration of exposure to bee venom extractor also had no significant effect on fecundity of experimental colonies. Thus the results provide the conclusion that the venom extraction process has no significant impact on egg laying ability of honeybees. Figure 19 shows the effect of different frame strength on fresh egg area of the colonies in all three seasons.

Table 20: Effect of bee venom extraction on fecundity (inch^2) during winter season of 2020-21

Frame strength	**Duration of exposure**	**Fresh egg area (Inch^2/colony)**		
		7 DAE 14-12-2020	**14 DAE 21-12-2020**	**21 DAE 28-12-2020**
6	**30 min**	15.67*	40.67	92.67
	45 min	17.67	42.00	83.33
	60 min	20.33	42.00	76.33
	0 min	29.00	44.67	95.33
	Mean	**20.67**	**42.33**	**86.92**
10	**30 min**	33.67	152.67	185.67
	45 min	32.00	160.67	186.33
	60 min	32.00	150.00	201.33
	0 min	37.00	163.00	212.33
	Mean	**33.67**	**156.58**	**196.42**

*Each data is mean of 3 replications
In winter extraction is done only once due to cessation of bee activity
SMW- Standard meteorological week
DAE- days after extraction
NS-Non-significant

Frame strength(A)	**CD (p=0.05)**	7.813	34.013	27.607
	SE(m)	2.551	11.106	9.014
Duration of exposure(B)	**CD (p=0.05)**	NS	NS	NS
	SE(m)	3.608	15.706	12.748
A X B	**CD (p=0.05)**	NS	NS	NS
	SE(m)	5.102	22.212	18.028

Table 21: Effect of bee venom extraction on fecundity (inch^2) during spring season of 2020-21

Frame strength	Duration of exposure	Fresh egg area (Inch^2/colony)					
		Extraction -1			Extraction- 2		
		7 DAE 02-03-2021	14 DAE 09-03-2021	21 DAE 16-03-2021	7 DAE 24-03-2021	14 DAE 31-03-2021	21 DAE 07-04-2021
6	**30 min**	131.33*	106.67	151.34	123.34	94.00	130.33
	45 min	118.33	137.67	153.67	123.33	119.33	111.00
	60 min	116.33	122.33	150.33	126.67	142.33	98.33
	0 min	139.67	140.67	158.34	140.67	151.33	122.67
	Mean	**126.42**	**126.83**	**153.42**	**128.50**	**126.75**	**115.58**
10	**30 min**	157.00	186.00	171.00	125.00	166.33	148.33
	45 min	161.00	178.33	181.00	169.00	167.33	163.67
	60 min	150.00	202.00	191.00	154.00	145.67	132.00
	0 min	159.00	194.33	228.33	163.00	173.00	169.67
	Mean	**156.75**	**190.16**	**192.83**	**152.75**	**163.08**	**153.42**

*Each data is mean of 3 replications
SMW- Standard meteorological week
DAE- days after extraction
NS-Non-significant

Frame strength(A)	**CD (p=0.05)**	NS	NS	NS	NS	NS	36.321
	SE(m)	13.392	22.988	24.755	20.737	18.512	11.86
Duration of exposure(B)	**CD (p=0.05)**	NS	NS	NS	NS	NS	NS
	SE(m)	18.94	32.51	35.009	29.326	26.179	16.772
A X B	**CD (p=0.05)**	NS	NS	NS	NS	NS	NS
	SE(m)	26.785	45.976	49.51	41.473	37.023	23.719

Table 22: Effect of bee venom extraction on fecundity (inch^2) during summer season of 2020-21

Frame strength	Duration of exposure	Fresh egg area (Inch^2/colony)					
		Extraction -1			Extraction- 2		
		7 DAE 04-05-2021	14 DAE 11-05-2021	21 DAE 18-05-2021	7 DAE 26-05-2021	14 DAE 01-06-2021	21 DAE 08-06-2021
6	30 min	113.33*	87.34	107.00	77.00	36.34	27.00
	45 min	91.33	91.00	85.67	72.33	33.67	25.00
	60 min	134.00	78.67	87.67	73.00	33.33	22.34
	0 min	129.67	115.67	95.00	90.34	43.67	25.67
	Mean	**117.08**	**93.17**	**93.83**	**78.17**	**36.75**	**25.00**
10	30 min	116.00	145.67	92.33	67.33	35.67	33.33
	45 min	110.00	115.33	95.34	91.00	38.67	25.67
	60 min	125.00	126.67	125.33	78.33	27.00	17.00
	0 min	132.33	151.33	108.00	94.67	50.33	26.67
	Mean	**120.83**	**134.75**	**105.25**	**82.83**	**37.92**	**25.67**

*Each data is mean of 3 replications
SMW- Standard meteorological week
DAE- days after extraction
NS-Non-significant

Frame strength(A)	CD (p=0.05)	NS	NS	NS	NS	NS	NS
	SE(m)	12.603	13.762	6.13	5.989	3.044	4.246
Duration of exposure(B)	CD (p=0.05)	NS	NS	NS	NS	NS	NS
	SE(m)	17.824	19.463	8.669	7.917	4.305	6.005
A X B	CD (p=0.05)	NS	NS	NS	NS	NS	NS
	SE(m)	25.207	27.525	12.259	11.196	6.088	8.492

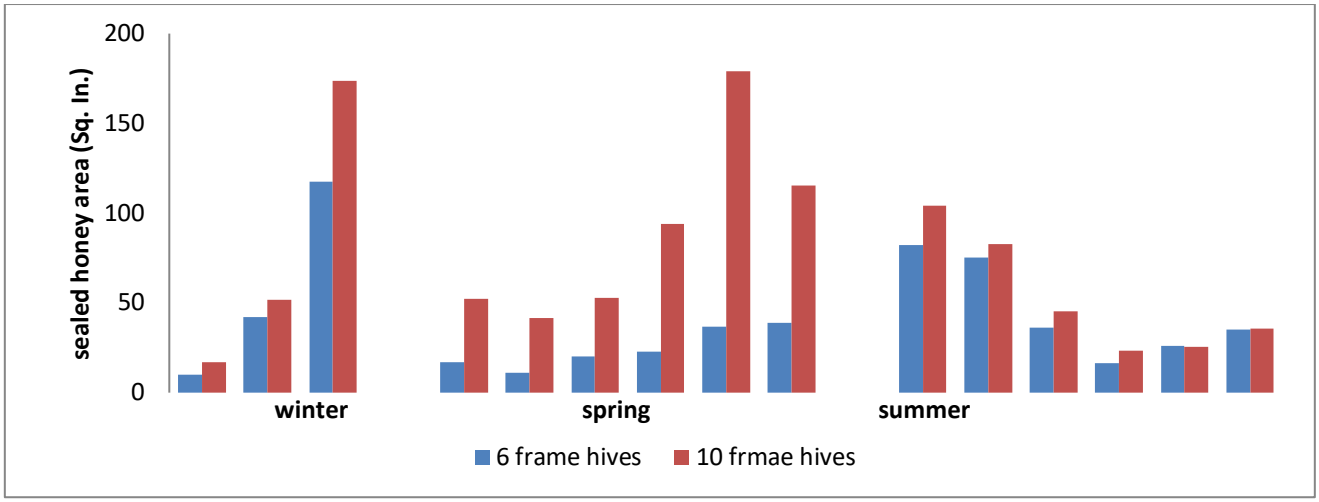

Figure 17: Effect of frame strength and different season on sealed honey storage of honey bee colonies during 2020-21

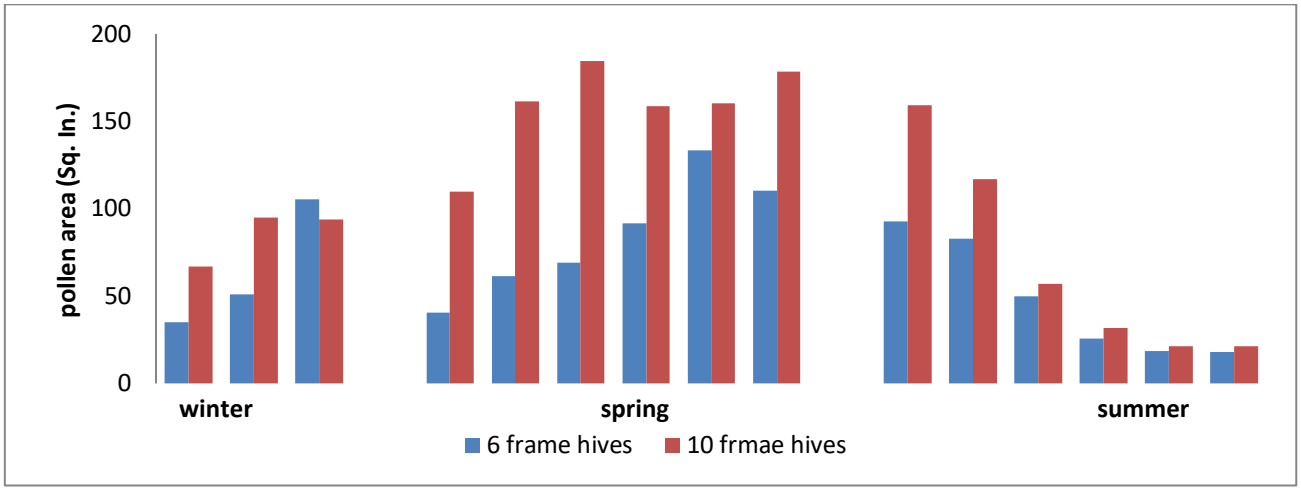

Figure 18: Effect of frame strength and different season on pollen storage of honey bee colonies during 2020-21

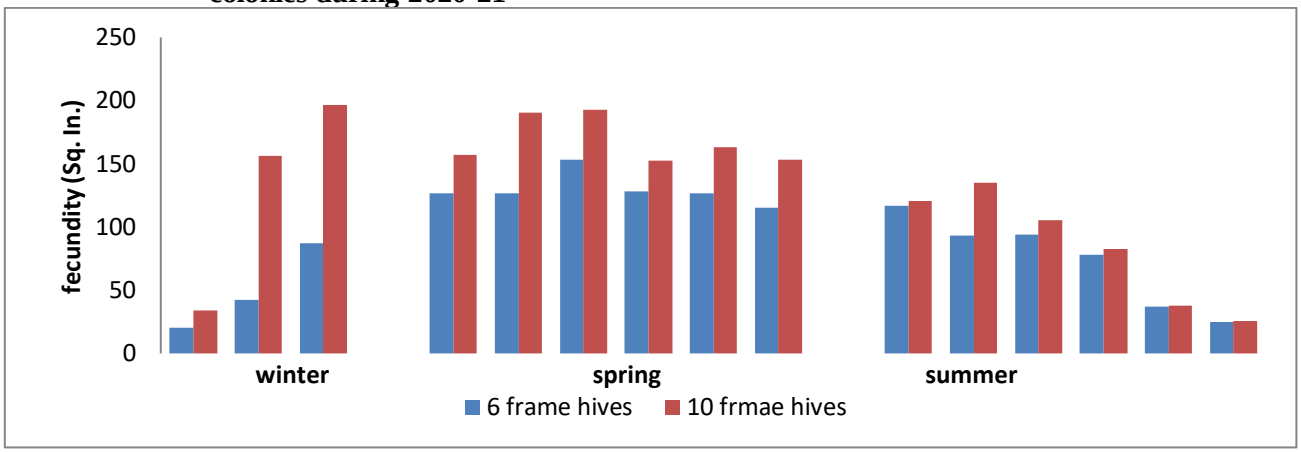

Figure 19: Effect of frame strength and different season on fecundity of honey bee colonies during 2020-21

CHAPTER-5

DISCUSSION

The current investigation entitled **"Seasonal variation in bee venom extraction and its effect on *Apis mellifera* L. colony performance"** was undertaken during winter, spring and summer season of 2020-21 at Apiary, Department of Entomology, CCS Haryana Agricultural University, Hisar. The findings and experimental results of the investigation are discussed in this following chapter under following subheadings below with information available on related aspects as meagre research has been done in this field.

5.1. Seasonal variation of bee venom quantity collected through bee venom extractor

A SB-BVC model bee venom extractor was used for bee venom extraction with the help of 9V battery power circulating in the steel wire grid over a glass plate where bee venom was deposited. Electric shock method used in this process created a defensive response in honey bees which came in contact with the electrocuted wire grid. Similar method for bee venom extraction had been used by Palmer (1961) with electrocuted steel wires running into agar gel sheets instead of glass plate and powered with variable voltages of electricity. Benton *et al.* (1963) electrocuted cupper or steel wire with 12V DC on nylon parchment taffeta stretched over a glass plate for collection of bee venom. Similar method of bee venom extraction was used by Dotimas and Hider (1987), Revataganv (2019), Brandeburgo (1992), Sanad and Mohanny (2013), Matysiak *et al.* (2011), Duran *et al*. (2011), Maulana *et al*. (2018), Junior *et al.* (2010), Mohanny(2015), Abrantes *et al.* (2017)

5.1.1. Bee venom quantity extracted

Bee venom from the hives was collected by placing the extractor at the bottom position with steel wire grids running over transparent glass plate. The range of bee venom quantity collected from each colony varied from 0.003g/colony to 0.052g/colony during entire investigation period, whereas variation within individual seasons were 0 g/colony to 0.052 g/colony in winter; 0.004 g/colony to 0.037 g/colony in spring; and 0.003 g/colony to 0.013 g/colony in summer. Bee venom extraction at the front of the hive became impossible during winter when maximum temperature of the become 17.5^0 C, as cold climate made it unfeasible for honeybees for their outdoor activities. Mohanny (2015) had used similar electric shock method to extract bee venom at top, middle and bottom positions of the hive with transparent, blue and mirror glass plate. Thus the amount of bee venom collected were 2.5776 g/colony (top position), 2.3462 g/colony (middle position), 2.1452 g/colony (bottom position), 6.0858 g/colony (transparent glass plate), 4.8877 g/colony (blue glass plate) and 9.3990 g/colony

(mirror glass plate). Similar observations had been taken by Funari (2001) in Brazil, with 0.0732±0.012 mg of bee venom was extracted whereas the amount of bee venom in the reservoir of Africanized bees were 0.117±0.015 mg of dry venom. Carniolan hybrids gave bee venom on an average of 0.38 g/colony and Italian hybrid gave 0.33 g/colony as per Omar *et al.* (2014)

The results of current investigation showed that maximum amount of bee venom was collected during spring (0.015 g/colony) followed by winter (0.012 g/colony) and summer (0.008 g/colony). Hussein *et al.* (2019) also observed similar results as maximum amount of bee venom was collected in spring (89.1 mg/colony and 80.7 mg/colony) followed by summer season (48.9 mg/colony and 39.8 mg/colony) and autumn season (15.9 mg/colony and 14 mg/ colony) from Carniolan and Italian hybrid colonies respectively. Sanad and Mohanny (2013) had reported that in bee venom collection Summer occupied the first rank producing the highest bee venom (0.161 g / day), followed by Autumn (0.116 g /day) and Spring (0.040 g / day) in Egypt. According to the experiment conducted by Omar (2017), maximum bee venom extracted during August (0.45 mg/colony) in 2012 and June (0.42 mg/colony) in 2013 whereas minimum amount of bee venom produced during February of 2012 (0.31 mg/colony) and 2013 (0.29 mg/colony). According to Omar *et al*.(2014) maximum bee venom was collected during August (0.40 g/colony) and minimum during February (0.3 g/colony). Mid-July was the best period for bee venom collection as per the experiment conducted in Poland by Rybak *et al*. (1995).

From the statistical analysis it was clear that hives with 10 frame strength produced more bee venom (0.016 g/colony) than 6 frame hives (0.009 g/colony). Fakim (1998) reported hives with 2-4 combs produced 0.026 g/colony on an average. According to El-saedy *et al.* (2016) bee venom amount has positive correlation with number of brood combs in a hive.

Hives exposed to bee venom extractor for 60 minutes (0.016 g/colony) produced maximum bee venom followed by hives exposed for 45 minutes (0.012 g/colony) and 30 minutes (0.007 g/colony). Thus bee venom amount is positively correlated with duration of exposure to the bee venom extractor. Nenchev and Stoichev (1997) reported increasing period of exposure to electrical stimuli from 60 minute to 90 minute doesn't produce more bee venom. Among the treatments used for bee venom extraction colonies with 10 frame strength exposed for 60 minutes produced maximum bee venom viz. 0.022 g/colony.

The electrical shock method which was used during the investigation for bee venom extraction from bees created stress in bees which led to natural defence response by the bees by stinging onto the glass plate. According to Onari *et al*. (2016) in this process of bee venom release, alarm pheromones (isopentyl acetate and 2-heptanone also releases in addition to bee venom. He also had an assumption that frequent harvesting of bee venom can put the

honeybees under stress, which can affect their potential to perform regular activities like brood rearing, egg laying, resource gathering, etc. This alarm pheromone can also have an effect on honey bee activities by involving in transmission of other pheromones of the colony (Kastberger *et al.*, 2008)

5.2. Seasonal variation in honeybee behavior due to the effect of bee venom extraction

5.2.1. Foraging behavior

5.2.1.1. Pollen foragers entering the hive per 2 minutes

Results from the experiment showed that number of pollen foraging bees was maximum during spring season followed by winter and summer (Table 3 and Figure 10). Spring season had maximum pollen foraging activity as this period acts as honey flow period and provides rich sources of pollen to bees. Furthermore there was significant difference between pollen foragers entering the hive per 2 minutes was due to different frame strength. 10 frame hives had been noticed with more pollen foragers entering the hives than 6 frame hives. This shows a positive correlation between frame strength and number of pollen foragers. In addition to this no significant difference was observed due to duration of exposure on population of pollen foraging bees, which signifies the electric shock method used for bee venom extraction has no effect on pollen foraging behaviour of bees.

5.2.1.2. Nectar foragers entering the hive per 2 minutes

From the table 4 and figure 10, it can be noticed that the population of nectar foragers remained significantly high in all three experimental seasons even before active brood rearing seasons perhaps to increase sealed honey storage for broods and remained significantly high during the late honey flow season probably to have enough honey storage for dearth period. Also, no significant difference between the populations of nectar foragers entering the hive per 2 minutes had been noticed due to frame strength except during 11th SMW, which showed more nectar foragers entering in 10 frame hives than 6 frame hives. But, duration of exposure had no significant effect on nectar foraging bees implying that nectar foraging activity doesn't get affected by bee venom extraction method implemented.

5.2.1.3. Number of bees going outside the hive per 2 minutes

Table 5 showed maximum number of bees going outside for foraging per 2 minutes during spring. The population increases from winter to spring and again decreased during summer (Figure 10). There was no significant difference observed due to frame strength or period of exposure among the foraging bees going outside from the hive. Thus we can conclude that electric shock method which is used for bee venom extraction has no significant effect on foraging behaviour of bees.

5.3. Seasonal variation in honeybee colony performance as a result of bee venom extraction

5.3.1. Sealed worker brood rearing

From the data taken on worker brood area during all the observations, it can be observed that worker brood area increased from winter to spring as spring is the active brood rearing period and decreased largely during summer due to lack of food sources (Figure 11). There was significant difference observed between 10 frame and 6 frame hives in case of worker brood rearing area. When means of worker brood rearing area of both frame strength were compared, it was noticed that 10 frame hives had more worker brood area than 6 frame hives. There was no significant effect of period of exposure observed on worker brood area. Thus it can be concluded that bee venom extraction had no significant effect on worker brood rearing of experimental colonies.

5.3.2. Sealed drone brood rearing

Figure 12 shows that there was increase in drone brood rearing area from winter to spring due to active brood rearing season, which later decreased largely as the dearth period approached. Drone brood rearing stopped at late spring in 6 frame hives whereas at summer in 10 frame hives. There was significant effect of frame strength observed on drone brood area and 10 frame hives had more drone brood area than 6 frame hives. This shows a positive correlation between frame strength and drone brood population. Further, no significant difference observed due to period of exposure to bee venom extractor on drone brood rearing area, which signifies that electric shock used for bee venom extraction is safe for honeybee colonies in drone rearing point of view. Observations on effect of bee venom on worker brood and drone brood rearing area has been taken by various scientists. No significant change in sealed brood area of the colonies had been observed by Omar (2017) and Skubida *et al*. (1995). But some other researchers had observed decrement in brood rearing area after bee venom extraction (Sanad and Mohanny, 2013; Onari *et al*., 2016, Nowar, 2016).

5.3.3. Brood survival

The colonies under investigation showed high brood survival rate irrespective of the frame numbers of the hives as shown in table 12. No significant difference of frame strength and duration of exposure to extractor had been observed when brood survival rate of colonies were concerned. Gholamian (2016) had also observed the same results that bee venom collection had no effect on survival of the colonies.

5.3.4. Mortality of bees

The electric shock of 9V power used for triggering bee venom production led to death of some honeybees as an immediate effect. To analyse the efficacy of an extractor this number

of dead bees should be minimum. The present investigation had also noticed some mortality of bees. The number of dead bees per colony ranged from 0 to 18.67 bees. Hives with 10 frames (4.72 worker bees/colony) showed more mortality than 6 frame hives (3.52 worker bees/colony, and hives exposed to bee venom extractor for 60 minutes (4.95 workers/colony) experienced maximum mortality followed by 45 minutes (4.17 workers/colony) and 30 minutes (3.25 workers/colony). Among the treatments, the descending order of dead bees was T_6 (5.61 workers/colony)> T_5 (5 workers/colony) >T_3 (4.28 workers/colony) >T_4 (3.56 workers/colony) >T_2 (3.34 workers/colony)>T_1 (2.95 workers/colony) making T_1 (2.95 workers/colony) safest for bee venom extraction. In case of seasons, spring (8.72 workers /colony) noticed with maximum mortality followed by winter (2.33workers/colony) and summer season (1.31 workers/colony). Seasonal effect on bee mortality was also taken under consideration by Sanad and Mohanny (2013) providing Summer season with the highest numbers of dead workers (50.3 workers / day) followed by Spring (40.9 workers /day) and Autumn (31.7 workers / day).

For a productive bee venom extraction, bee venom quantity should be higher with minimal death of bees. This is achieved by correlating bee venom amount with bee mortality after respective extraction. Hives with 10 frames showed 4.72 workers as dead and produced 16 mg bee venom/colony whereas hives with 6 frames had 3.52 dead bees and produced 9 mg bee venom/ colony. From the figure 16 we can notice that 10 frame hives were more productive and safer as the gap between both the parameter was more. Similarly in case of period of exposure; although mortality of bees were higher (4.95 bees/colony) in hives exposed for 60 minutes, it produces maximum amount of bee venom (16.5 mg/colony) (Figure 15). The difference between bee mortality and bee venom amount was the widest in case of 60 minute duration of exposure making it the more productive and safe for both bee venom and bee mortality point of view. Similar observations for different treatments details has provided T_6 (5.61 dead bees/colony and 22 mg bee venom/ colony) as the most productive and safest treatment for bee venom extraction (Figure 13). In case of different seasons, although spring has been witnessed with maximum bee venom quantity extracted (15 mg/colony), it was also recorded with maximum number of dead bees (8.72 worker bees/colony). But winter season has been noticed with a wider gap between bee venom amount (12 mg/colony) and bee mortality (2.33 worker bees/colony) being the safest and most productive season for bee venom extraction compared to the other two (Figure 14).

5.3.5. Honey storage

Sealed honey areas in colonies under investigation increased gradually during winter to provide enough food to rearing brood in active brood season as shown in table 14. During

spring also sealed honey areas increased regularly to fulfil the demand of rearing brood but in summer it started decreasing due to lack of food sources as mentioned in table 15 and 16. Significant differences had been noticed among the colonies during spring season due to frame strength probably because of active brood rearing season and availability of sufficient nectar sources. Hives with 10 frames showed more sealed honey area than hives with 6 frames, which indicates sealed honey area is positively correlated with bee strength of the hives. No significant difference was observed in sealed honey areas of the colonies due to period of exposure implies electric shock method of bee venom extraction has no impact on honey storage of honeybee colonies. Similar results have been concluded by Bahreini *et al*. (2000) and Gholamian *et al.* (2006). In contrast negative impact due to electric shock method of bee venom collection on honey storage/yield has been noticed by Skubida *et al*. (1995), Zhou *et al.* (2003) and Ghazala and Taha (2014).

5.3.6. Pollen storage

The pollen area of the experimental colonies increased from winter to spring and decreased from spring to summer (Figure 18). Pollen area reaches its peak during spring season due to availability of pollen sources in honey flow season. Significant difference between 10 frame hives and 6 frame hives was seen regarding pollen storage area of the colonies during winter and spring probably to store and fulfil the demand of developing broods. Frame strength of the colonies had positive correlation with pollen storage as 10 frame hives had more pollen area than 6 frame hives. Whereas duration of exposure had no significant effect on pollen storage which concludes that electric shock method of bee venom extraction doesn't affect pollen storage of the experimental colonies. But Skubida *et al*. (1995) had noticed negative impact of bee venom collection on pollen storage of the colonies. As per the investigation done by Khattab (2000), pollen areas of venom extracted colonies decreased from initial recorded observation before the extraction.

5.3.7. Fecundity

The growth pattern observed in case of fresh egg area was similar to that of worker brood, drone brood and pollen storage of the experimental colonies. Area with fresh eggs increased to maximum during spring season due to increased brood rearing at that period. Significant difference due to frame strength observed in case of fecundity during winter season, with the fact that frame strength had been positively correlated to the area with fresh eggs (Figure 19). 10 frame hives had more fresh egg area than 6 frame hives. Electric shock extraction of bee venom had no impact on fecundity of the colonies as no significant difference due to period of exposure was observed after statistical analysis. Onari *et al*. (2016) had noticed decrease in uncapped brood area in colonies treated with bee venom harvest than the untreated ones.

CHAPTER-6

SUMMARY AND CONCLUSION

The current investigation entitled **"Seasonal variation in bee venom extraction and its effect on *Apis mellifera* L. colony performance"** was carried out during winter, spring and summer season of 2020-21 at Apiary, Department of Entomology, CCS Haryana Agricultural University, Hisar. The findings of the experiment are summarised below in this chapter

The present investigation was carried out to know the effect of various factors like frame strength, period of exposure and seasons on bee venom quantity extracted and to know the effects of electrical method of venom extraction on behaviour and performance of honey bee colonies, by using a bee venom extractor of model SB-BVC (manufactured by DPS Tech Smart Pvt. Ltd., New Delhi). Ultimate goal was to record the variation in bee venom amount extracted due to different factors imposed and to analyse the efficacy of the apparatus in reference to honey bee colony behaviour and colony growth parameters.

6.1. Seasonal variation of bee venom quantity collected through bee venom extractor

6.1.1. Bee venom quantity extracted

As per the results attained after the investigation, maximum bee venom quantity is obtained during spring season (0.015g/colony) followed by winter (0.012 g/colony) and summer (0.008 g/colony). Colonies with 10 frames produced more bee venom (0.016 g/ colony) compared to colonies with 6 frames strength (0.009 g/colony).The descending order of different treatments according to the bee venom quantities extracted is) T_6(0.022 g/colony)> T_5(0.015g/colony) >T_3(0.011gm/colony) >T_4(0.010g/colony) >T_2(0.009g/colony) >T_1(0.005g/colony). Hives exposed to bee venom extractor for 60 minutes (0.016 g/colony) produced maximum bee venom followed by hives exposed for 45 minutes (0.012 g/colony) and 30 minutes (0.007 g/colony).

6.2. Seasonal variation in honeybee behavior due to the effect of bee venom extraction

6.2.1. Foraging behavior

Significant difference in pollen and nectar foraging behaviour of the experimental colonies were observed during late spring period due to different frame strength, but no significant difference was observed due to duration of exposure to the bee venom extractor. This result concluded that foraging activities of bees like pollen and nectar foragers entering and leaving the hive was not affected by the electrical stimulation given to the bees.

6.3. Seasonal variation in honeybee colony performance as result of bee venom extraction

6.3.1. Sealed worker brood rearing

Different frame strength of experimental colonies had put significant effect on worker brood rearing and had been noticed that 10 frame colonies had more worker brood area than 6 frame colonies. Whereas electrical stimulation used for venom extraction had no significant effect on worker brood rearing of colonies as no significant difference observed due to period of exposure to the extractor.

6.3.2. Sealed drone brood rearing

Significant difference between drone brood area of 10 frame and 6 frame hives was observed with more drone brood area under 10 frame hives. Duration of extraction hadn't created any significant different in drone brood area of the colonies making the electrical extraction safe for brood rearing activity of bees.

6.3.3. Brood survival

Both, different frame strength and period of exposure had produced any significant difference in brood survival rates of the colonies. Thus it can be concluded that bee venom extraction has no effect on brood survival of colonies.

6.3.4. Mortality of bees

Mortality of bees were maximum during spring (8.72 workers/colony) followed by winter (2.33 workers/colony) and summer (1.31 workers/colony), which makes summer is the safest season for bee venom extraction. Numbers of dead bees were more in 10 frame colonies (4.72 workers/ colony) than 6 frame colonies (3.52 workers/ colony). Hives exposed to bee venom extractor for 60 minutes (4.95 workers /colony) experienced maximum mortality followed by 45 minutes (4.17 workers/colony) and 30 minutes (3.25 workers/ colony). In case of different treatments, same trend follows as in bee venom quantity extracted viz. T_6 (5.61 workers/colony)> T_5 (5 workers/colony) >T_3 (4.28 workers/colony) >T_4 (3.56 workers/colony) >T_2 (3.34 workers/colony)>T_1 (2.95 workers/colony). From above mentioned results it can be concluded that frame strength of 6, 30 minutes of exposure and summer season is the safest period of bee venom extraction as it experiences minimum mortality. Further, when comparison between bee venom quantity and respective bee mortalities were made, winter season, 10 frame hives and 60 minutes of exposure to extractor was proved to be best for both productivity and safety point of view.

6.3.5. Sealed honey storage

Significant difference in sealed honey storage was observed during spring season due to different frame strength with more area in 10 frame hives, but no significant difference was observed due to period of exposure making the bee venom extractor safe for use.

6.3.6. Pollen storage

Pollen storage area of experimental colonies was significantly affected by different frame strength during winter and spring with more area observed in 10 frame hives; whereas electrical shock method had no significant impact on pollen area as period of exposure to extractor hadn't put any impact on it.

6.3.7. Fecundity

Fecundity of experimental colonies was affected significantly during the winter season before the initiation of active brood period with more fresh egg area in 10 frame hives. Further no impact of period of exposure was observed o fecundity of colonies making the extractor safe for bee venom extraction with respect to egg laying capacity of the colonies.

6.4. Conclusion

According to the present investigation carried out, extraction of bee venom during both dearth and honey flow period can provide a year round income to bee keeper. Bee venom amount extracted changed abruptly whereas the change in bee mortality of respective colonies was less steep. Thus from safety and productivity point of view, colonies with higher strength and colonies exposed to longer duration to the bee venom extractor will produce higher amount of bee venom. In addition to this, low rate of mortality due to the electrical stimulation provided that the apparatus used viz. SB-BVC model manufactured by DPS tech smart pvt. Ltd, New Delhi, is safe for honey bees. The results derived from the investigation regarding the colony behaviour and colony growth characters in routine activities were shown to be not affected by electrical method of extraction. This increases the effectiveness of bee venom extractor used in Haryana agro climactic conditions.

CHAPTER-7

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ABSTRACT

Title of thesis	:	Seasonal variation in bee venom extraction and its effect on *Apis mellifera* L. colony performance
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Admission number	:	2019A48M
Title of degree	:	Master of Science
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Key words: Bee mortality, brood rearing, efficacy, foraging behaviour, honey, Honeybee venom, pollen, safety, venom extraction

The present investigation entitled "Seasonal variation in bee venom extraction and its effect on *Apis mellifera* L. colony performance" was carried out at Apiary, Department of Entomology, CCS Haryana Agricultural University, Hisar during winter, spring and summer season in the year 2020-2021. The effect of various factors like frame strength, period of exposure and season on bee venom extraction and further impact of electrical bee venom extraction on honey bee colony behaviour and colony performance were examined. It was observed that 10 frame hives (0.016 g/colony) produced more bee venom than 6 frame hives (0.009 g/colony). Hives exposed for 60 minutes (0.016 g/colony) extracted more bee venom than 45 minutes (0.012) and 30minutes (0.007 g/colony) of exposure. Spring season (0.015 g/colony) noticed with maximum bee venom collection followed by winter (0012 g/colony) and summer (0.008 g/colony). Similar trend was observed in case of bee mortality due to electrical bee venom extraction. 10 fame hives (4.72 workers/colony) noticed with more number of dead bees/colony than 6 frame hives (3.52 workers/colony). Colonies exposed for 60 minutes (4.95 workers/colony) experienced more death than 45minutes (4.17 workers/colony)and 30 minutes (3.25 workers/colony) of exposure. Spring season (8.72 workers/colony) was noticed with maximum bee mortality followed by winter (2.33 workers/colony) and summer (1.31 workers/colony). Among the treatments also T_6 (colonies with 10 frame and 60 minutes of exposure) produced more bee venom 0.022 g/colony) and noticed with maximum mortality (5.61 workers/colony) than other treatments. When bee venom amount was compared in relation with bee mortality, it was observed that colonies with highest strength (10 frames), longer duration of exposure(60 minutes) and winter season were the most productive and the safest for bee venom extraction compared to respective other factors due to the widest difference between bee venom amount and bee mortality. Significant difference was also observed because of different frame strengths in foraging behaviour, worker brood rearing, drone brood rearing, fecundity, sealed honey storage and pollen storage of the experimental colonies, although, duration of exposure didn't have any significant effect on colony behaviour and performance parameters. This rendered the evidence of safety and efficacy of the bee venom extractor apparatus used in the investigation.

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I, **N. Aparna**, Admission No. **2019A48M** undertake that I give copy right to the CCS Haryana Agricultural University, Hisar of my thesis entitled "**Seasonal variation in bee venom extraction and its effect on *Apis mellifera* L. colony performance**". I also undertake that patent, if any, arising out of the research work conducted during the programme shall be filed by me only with due permission of the competent authority of CCS HAU, Hisar.

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